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6209

Roentgen-ray Study on Non-permeability to Thorium Dioxide of Placenta of Albino Rat.*

LEON J. MENVILLE AND J. N. ANÉ.

From the Radiological Division, Department of Medicine, Tulane University.

Experimental work on the permeability of the placenta to certain dyestuffs has been reported by a number of investigators. Wislocki[1] reports that when phenolsulphonephthalein is injected into the peritoneal cavity of a guinea pig fetus it is absorbed and passes slowly from the fetal blood stream through the placenta into the maternal blood stream and is excreted by the maternal kidney. A similar injection of trypan blue is also absorbed from the peritoneal cavity of the fetus, but is not transmitted by the placenta to the maternal organism. According to Wislocki's observations the placenta is incapable of transmitting an inert foreign colloid from the fetus to the mother. Shimidzu[2] injected pregnant rats subcutaneously between the scapulae, using 23 different dyes, 8 basic and 15 acidic. He found that the placenta was permeable to all of the basic dyes, and only 8 out of the 15 acid dyes penetrated the placenta; also that the permeable dye solutions were slightly colloidal or non-colloidal in character, with the exception of Biebrich's scarlet, a highly colloidal dye. Dyes largely in the colloidal state do not, with rare exceptions, penetrate the placenta. These experiments were made on animals either at operation or after death.

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* Aided by a grant from the David Trautman Schwartz Research Fund.

[1] Wislocki, G. B., *Bull. Johns Hopkins Hosp.*, 1921, **32**, 93.

[2] Shimidzu, Y., *Am. J. Physiol.*, 1922, **62**, 202.

We were unable to find in the literature reports of experiments on the permeability of the placenta to colloidal substances opaque to the roentgen ray. We appreciated that but few colloidal substances have been found which penetrate the placenta because of the size of the particles in these substances. We used thorotrast, a colloidal thorium dioxide, miscible in all proportions with body fluids. We hoped that its particles of thorium would be small enough to penetrate the placenta, as in the instance of Biebrich's scarlet, and thereby visualize the reticulo-endothelial system of the fetus.

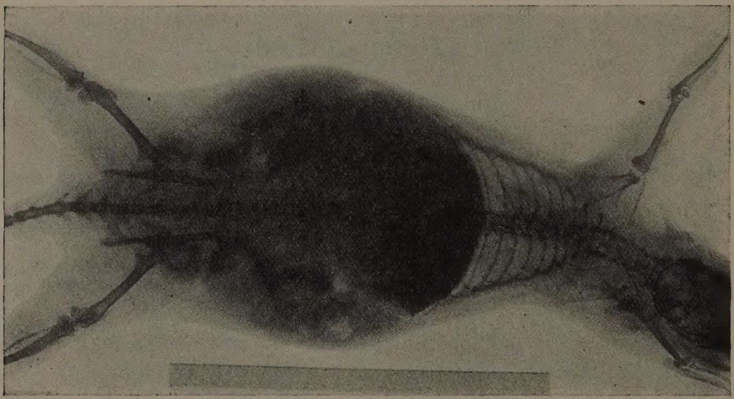

FIG. 1.

Pregnant rat, showing the visualized placentas. The liver and spleen of fetuses not visualized.

We used four pregnant albino rats, at or near full term. Three were injected with 1 cc. of thorotrast, and shortly after were roentgenographed, the liver and spleen being clearly visualized. The livers and spleens of the fetuses did not show the presence of thorium at that time. These rats were roentgenographed several times before delivery, with the same results. Roentgenograms were also made of the newborn shortly after birth, and we were unable to detect thorium in their livers or spleens. From the roentgenograms of these 3 rats there was no roentgen evidence that the thorium had reached the placenta. We then injected 1.5 cc. of thorotrast in the tail vein of the 4th pregnant rat, and a roentgenogram, made shortly after, clearly showed the liver, spleen, and 9 placentas, but the thorium, as far as we could observe, had not penetrated the placentas. This rat died 24 hours after injection, and through the kindness of Dr. William Harris, of the Pathological Department, Tulane University, histopathologic studies were made of the placentas, livers, and spleens of the fetuses. The placentas showed the presence of thorium, but none was found in the fetuses.

6210

Microscopic Changes Produced in Tissues of Animals Injected with Thorium Dioxide for Spleno-hepatography.

WILLIAM H. HARRIS AND A. V. FRIEDRICHS.

From the Department of Pathology, College of Medicine, Tulane University.

Many reports have recently appeared upon the results of the intravenous injection of colloidal thorium dioxide (Thorotrast-Heyden) in the experimental animal, most of them demonstrating tolerance and lack of toxicity even in large doses. Huguenin and Nemours[1] state that 18 cc. of thorotrast were tolerated by the rabbit without any after-effects. Later, however, with Albot,[2] they described the production of hepatitis and cirrhosis in rabbits with doses of 15 to 20 cc. Bauman and Schilling[3] found that 1 cc. administered intravenously in rabbits, was necessary for contrast shadows of the spleen and liver but that 3 cc. twice daily up to 12 cc. gave more distinctive shadows, with no ill effects. Fifteen minutes after injection, glossy colorless grains could be observed in the various stellate (Kupffer) cells and also in the reticulum cells of the spleen. Twenty-four hours later, such cells were swollen and filled with fine and coarse, glossy granules. After 14 days the same approximate condition existed. Popper and Klein[4] injected rabbits and dogs up to 8 times the quantity recommended for the human subject. They state it was harmless and that the blood was not affected. Lambin,[5] however, found that 5 cc. per kilo in rabbits necessary for liver visualization caused anemia whereas 1 cc. per kilo, sufficient for spleen visualization, caused an erythroblastic reaction, without producing anemia. When a pronounced anemia was produced, the recovery was spontaneous. Kadrnka[6] reports haematuria in animals subsequent to intravenous injections of thorium dioxide. Thus conflicting observations have been recorded.

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[1] Huguenin, R., and Nemours, A., *Rev. de Path., comp. et d'Hyg. gen.*, 1931 **415**, 480.

[2] Huguenin, R., Nemours, A., and Albot, G., *Comp. rend. de Seances d. Biol.*, 1931, **35**, 879.

[3] Bauman, H., and Schilling, C., *Klin. Wschr.*, 1931, **27**, 1249.

[4] Popper, H., and Klein, E., *Muench. med. Wschr.*, 1931, **43**, 1829.

[5] Lambin, P., *Comp. rend. Soc. Biol.*, 1931, **28**.

We injected 53 white rats in collaboration with radiological observations of Drs. Menville and Ané, with variable results as regards tolerance. The very moderate dosage was from 0.2 to 1 cc. introduced in the tail vein. In the human subject as much as 50 to 75 cc. has frequently been injected. About 4% died within 48 hours. Those surviving this period with the exception of the pregnant females continued to live after 3 months in apparently good health. The animals studied histologically included those dying early as well as those surviving and in good condition, sacrificed with ether anesthesia.

In the animals dying from the injection within 48 hours, the following microscopic changes were noted: The liver showed cast-like masses of small thorium granules in the sinusoids and marked parenchymatous degeneration with fatty changes were present in the hepatic cords. In the kidneys, larger glossy thorium granules were found in the glomerular capillary tufts. The renal tubuli showed in areas, occlusion of the lumen from swelling of the renal epithelium while others showed extensive parenchymatous degenerative changes. The spleen showed extensive collection of thorium granules which had replaced the splenic tissue in much of its structural extent. The deposits were present in the splenic corpuscles and the splenic pulp. The material was not phagocytized. In one animal in which the lungs were visualized the capillaries of the alveolar walls were laden with glossy thorium granules.

In the animals in apparent good health the changes noted in general were those described by most observers, *i. e.,* the selective reaction of the reticulo-endothelial cells, especially of the liver and the spleen, which structures apparently represent the accumulating foci of the thorium injections. It does not seem entirely accurate to state, however, that the selective affinity of the reticulo-endothelial system would account solely for the extensive and consistent splenohepatic accumulations. It is true that the bone marrow does show some collection of this material but the vascular supply of the lungs, kidneys, lymphatic glands and other structures forming a part of the Aschoff system is seldom visualized.

The cellular response produced is somewhat analogous to that seen in typhoid fever. The displacement of splenic structure, the apparent invasion and destruction of areas of hepatic cord epithelium and the deposition of the material in the portal tracts is, however, a more definite histo-pathologic change than simply a phagocytic reaction on the part of the reticulo-endothelial system.

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[6] Kadrnka, S., *Radiology,* 1932, **18**, 371.

It is possible that the white rat presents a somewhat different response than that of other animals. It is also possible that the thorium preparations vary slightly in the character of their constituents.

6211

Histo-pathological Effects of Thorium Dioxide on Lymphatic Glands of Animals Visualized by the Menville-Ané Method.

WILLIAM H. HARRIS.

*From the Department of Pathology, College of Medicine, Tulane University.**

The method of radiological visualization of the lymphatic glands by the injection of colloidal thorium dioxide (thorotrast) was described by Menville and Ané.[1] In this work the material was introduced subcutaneously in the area of the perivascular lymph spaces. The subsequent drainage to the lymphatic glands produced visualization not alone of the glandular structure but also, at times, the lymphatic vessels (Fig. 1). Through this means, the opportunity

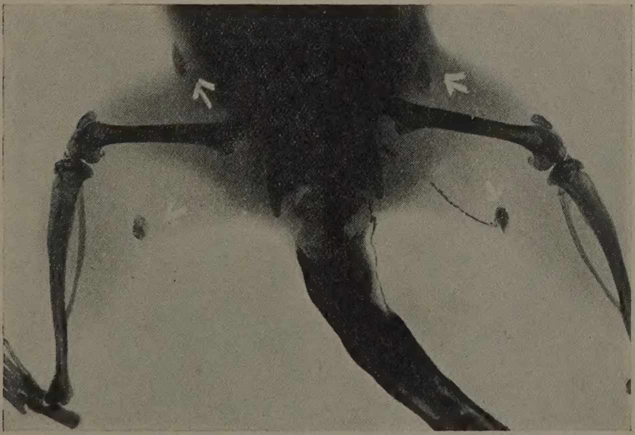

FIG. 1.

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* Aided by a grant from the David Trautman Schwartz Research Fund.

[1] Menville, L. J., and Ané, J. N., *J. Am. Med. Assn.*, 1932, **98**, 1796.

has been afforded of studying the host response of the lymphatic glands thus visualized, in 5 white rats, 6 rabbits, and 3 dogs.

The tissue reactions produced have been, in certain instances, unusual and unique in their aspect. It is to be noted that aside from the injected material, these animals have been visualized with the X-ray. While it is presumed that the changes noted are due to thorium alone because of the very slight exposure to the X-ray, nevertheless the combination of these factors is to be considered. Further animal experiments separating these activities are under way.

In observations heretofore made upon both animals and the human subjects injected intravenously, the phagocytosis of thorium by cells of the reticulo-endothelial system forms the outstanding description. Huguenin, Nemours and Albot[2] describe, however, the production of a hepatitis and cirrhosis in the rabbit presenting thereby a connective tissue reaction apparently responding to hepatic cell injury.

In the animals of these experiments injected subcutaneously and the lymphatic glands thereby visualized, there occurs a distinctly stimulating reaction upon the connective tissues or stromal unit of the glands, with loss of the lymphoid structure. At times, associated with the connective tissue increment there occurs an enlargement and evident stimulation of the cortical nodules, indicated especially by the great increase of the germinal or keim centers. The

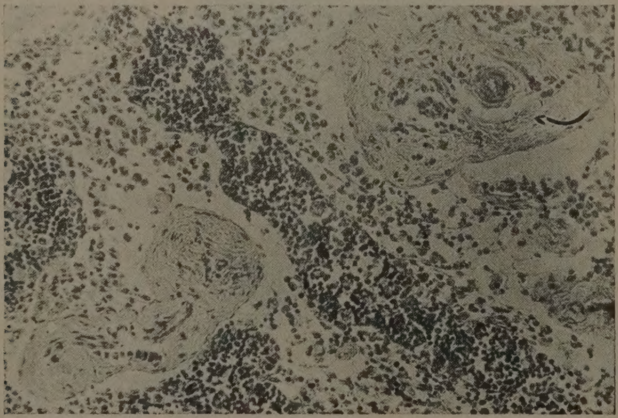

FIG. 2.

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[2] Huguenin, R., Nemours, A., and Albot, G., *Comp. rend. de Seances, d. l. Soc. d. Biologie*, 1931, **35**, 879.

feature of unusual interest is the perivascular connective tissue proliferation (Fig. 2) which continues to increase concentrically about the blood vessels. Phagocytic cells which contain thorium can be seen within the lymph sinuses. A more advanced picture of such changes presents complete loss of the parenchyma with connective tissue replacement in which the lymph spaces are clearly shown and at times appears dilated (Fig. 3). Occasionally masses of phagocytic cells containing thorium show a surrounding encapsulation of connective tissue. At the site of local inoculation, masses of foreign body giant cells or macrophages are found heavily laden with thorium granules.

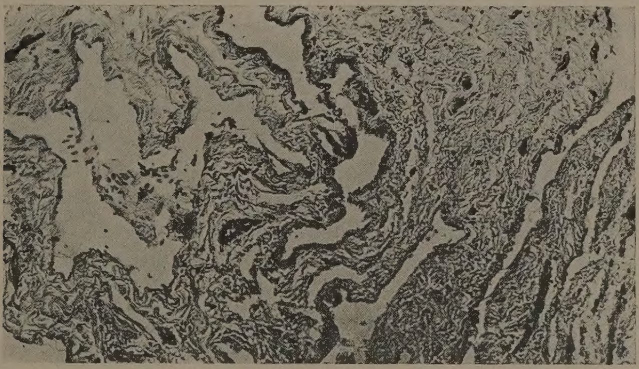

FIG. 3.

The biological effects produced upon lymphatic glands visualized by the subcutaneous injection of thorium are unusual in character and differ from those resulting from radium or X-ray.[3] As this resultant activity may represent changes of subsequent important significance in other studies, the results have accordingly been presented.

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[3] Wood, F. C., A Text-book of Path., Delafield and Prudden, 1931; William Wood and Co., N. Y., 14.

6212

Intraperitoneal Injections of Pneumococcus in Animals Primarily Receiving Leucocytic Stimulating Antigens.*

HERBERT J. SCHATTENBERG AND WILLIAM H. HARRIS.

From the Department of Pathology, College of Medicine, Tulane University.

In a previous communication[1] we reported the difference in leucocytic response to the injection in animals of various antigenic substances. The differences were of a marked character, not only for the different antigens used but also for the route of injection chosen. Killed cultures of *B. typhosus* and *Staph. aureus* injected intraperitoneally resulted in the formation of the highest leucocytic increase. The peak of the rise appeared in from 6 to 8 hours. We also called attention to the phenomenon of local leucocytosis as a possible misleading factor in making such observations.[2] Some allusion to this feature had previously been made by Garrey and Butler.[3, 4]

Steinberg[5] reports that 4 intraperitoneal injections of heat killed *B. coli* at daily intervals, protects 65% of animals against an artificially produced peritonitis caused by a living *B. coli* suspension administered on the 5th day. Gay and Clapole[6] have shown that the "carrier state" in rabbits can be prevented if previously immunized rabbits are injected with a live suspension of *B. typhosus.* Their experiments show that there is a much greater leucocytic response when immunized rabbits are injected than when normal rabbits are used and in the latter case the "carrier state" cannot be prevented.

To ascertain the protective influence of induced leucocytosis, 47 white mice received intraperitoneal injections of the following antigenic substances for the purpose of producing leucocytic reactions in approximately 6 hours as controlled by the previously reported experiments:[7] (1) Suspension of killed *B. typhosus.* (2) Suspension of killed *Staphylococcus aureus.* (3) Detoxicated suspension of killed pneumococcus. (4) Sterile milk.

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* Aided by a grant from the David Trautman Schwartz Research Fund.

[1] Harris and Schattenberg, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 265.

[2] Schattenberg and Harris, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 269.

[3] Garrey, W. E., and Butler, Virginia, *Am. J. Physiol.*, 1929, **90**.

[4] Garrey, W. E., and Butler, Virginia, Reprinted from *Proceedings of the Staff Meetings of the Mayo Clinic*, 1929, **4**.

[5] Steinberg, Bernhard, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 16.

[6] Gay, F. P., and Claypole, E. J., *Arch. Int. Med.*, 1914, **14**, 662.

[7] Harris and Schattenberg, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 265.

After 6 to 8 hours these animals were injected intraperitoneally with lethal doses of Pneumococci types I, II, and III. These cultures were grown in Avery's medium and suspended in saline solution to produce a suspension containing approximately 300,000,000 organisms per cc. The amounts given, varying from 0.1 to 0.25 cc.,. were adjusted so that death of the animals could be controlled and occurred at periods varying from 3 to 24 hours.

The animals in the various series received a respective antigenic injection approximately 6 hours previous to the administration of the infecting dose of the pneumococcus. In one series, 2 antigenic doses were given at 24 hour intervals and an infecting dose 6 hours following the last injection. Control animals for the infecting dose were run with each series and the effects of the antigenic injection were also noted in other control animals.

While in certain instances some of the supposedly protected animals survived for several hours longer than the infection control animal, at other times the control lived somewhat longer than those that had received the antigenic dose or doses.

Our procedure does not conform to that used by Steinberg in that our infecting microorganism was more virulent and that repeated injections to produce a hyperleucocytic immunity were not administered.

The results of our experiments would indicate that in white mice, the injections of various antigenic substances employed as stimulators for a leucocytic increase, failed to show any evidence of protection to this animal against the production of fatal pneumococcal peritonitis.

6213

Effects of Toxic and Non-toxic Doses of Thorium Dioxide in Various Animals.

C. J. TRIPOLI AND E. v. HAAM.

From the Departments of Pathology of Charity Hospital and Louisiana State University Medical Center.

The value of the method of roentgenographic visualization of the liver and spleen in the human is, in our opinion, dependent only upon the possible dangers involved in the intravenous administration of stabilized colloidal suspensions of thorium dioxide (Thorotrast).

We have undertaken a series of experiments to determine the histopathological changes in the tissues of animals that have been given various intravenous doses of thorium dioxide over varying periods of time.

Seventy-nine animals, comprising 4 dogs, 15 albino rats, 18 guinea pigs, 20 white mice, and 22 rabbits, have been used in this work. All animals were injected with doses varying from that which permits good visualization (0.8 gm. per kg. body weight) to 10 times this dose. An additional number of animals were used as controls in each experiment. The dogs and rabbits were injected intravenously, whereas the guinea pigs, white mice and rats were injected by the intracardiac route. The complete dose was given over a period of 3 days, one-third of the total dose each day. The animals were then killed at intervals of one day, one week, one month and 6 months following the injection of both small and large doses of the thorium preparation.

The following observations were made: All animals which received 0.8 gm. per kg. body weight showed no changes in appetite, skin, body weight, susceptibility to infections or propagation of the species. The animals were all living and well up to the time of being sacrificed.

Several animals receiving 10 times the smaller dose (8.0 gm. per kg. body weight) showed toxic effects. Three guinea pigs and 2 rabbits lost considerable weight and were apparently ill for 2 weeks, but recovered and were killed at the end of one month. Two rabbits, one guinea pig and 3 white mice died within one to 3 days following the last injection. Thus 69 animals survived throughout the time of the experiment without any notable changes.

These surviving animals were sacrificed and autopsied at varying intervals following administration of the drug with the following gross findings: No gross changes nor any evidence of any type of hemorrhage was noted in animals receiving 0.8 gm. per kg. body weight, irrespective of time elapsing between final administration of the drug and death, ranging from one day to 6 months. In those animals receiving 10 times the smaller dose, the liver and spleen were paler than normal. In none was there any hemorrhage present. Two rabbits and one mouse of this series were pregnant. The embryos were living at the time of autopsy. In some of the animals intracardially injected, the mediastinal lymph nodes were markedly enlarged.

Histopathological study revealed the following:

1. Animals which received doses of 0.8 gm. per kilo.

a. Spleen. With such animals sacrificed at the end of 24 hours, a few thorium particles were found free in the finer capillaries. Reticulo-endothelial cells were found already containing the thorium particles. At the end of one week, no free thorium was found, but many reticulo-endothelial cells contained the metal. At the end of one month, fewer reticulo-endothelial cells were found containing the thorium, much reduced in amount. At the end of 6 months, a very few reticulo-endothelial cells scattered throughout the organ contained the thorium particles. No evidences of hemorrhage or fibrosis were noted in any instance.

b. Liver. The liver as regards the free particles of thorium and the reticulo-endothelial cells presented in all cases the same picture as described for the spleen at the end of corresponding time intervals. The liver cells of early sacrificed animals occasionally contained a few particles of thorium, but in animals later sacrificed, thorium was not present.

c. None of the animals receiving the small dose showed any thorium in any organ other than the liver and spleen. No histopathological changes could be demonstrated in any organ of any of the animals as compared to apparent healthy control animals.

2. Animals which received 8 gm. per kilo.

a. Spleen. Many thrombi of thorium particles were present in the capillaries and free particles were found in the splenic pulp at the end of one day. Minute areas showing beginning necrosis were present. At the end of one week, the reticulo-endothelial cells had engulfed all of the thorium and none was found free. No necrosis or fibrosis was present. Guinea pigs and rabbits showed the thorium particles in the center of the Malpighian bodies, whereas the mice and white rats had the thorium in the cells of the periphery of the lobule. At the end of one month, the only change in the picture was the presence of a smaller amount of thorium as estimated by visual means. At the end of 6 months, certainly over one-half of the thorium had disappeared from the reticulo-endothelial cells of the spleen.

b. Liver. The changes were quite similar to those described for the spleen, there being thrombi of thorium particles present in the finer capillaries and marked parenchymatous degeneration of the liver cells, with no actual necrosis noted. At the end of one month a few thrombi remained, but retrograde changes of the liver cells were no longer noted, all the thorium being then found in the reticulo-endothelial cells. Animals sacrificed at the end of 6 months showed that a great deal of the thorium had disappeared from the cells. No hemorrhage or fibrosis was noted at any time.

c. Kidneys. White mice killed one week after injection showed exudate and thickening of the glomerular capsule with small thrombi in the glomerular loops. Guinea pigs, rabbits and albino rats showed degeneration of the proximal convoluted tubules. Animals sacrificed one month following injection show no renal lesions.

d. Animals showed thorium particles present in the reticulo-endothelial cells of the bone marrow, adrenal glands and lung.

e. Brain, heart, vascular system, pancreas, gastro-intestinal tract, genito-urinary organs, placenta and embryonic tissues showed no evidence of thorium particles except for renal glomerular thrombi as previously noted in animals sacrificed one week after injection. The smaller animals receiving accidental mediastinal dosages while attempting intracardiac injections showed thorium in the pericardium and mediastinal lymph glands.

Summary. 1. The reticulo-endothelial cells, particularly in the liver and spleen, have been shown constantly to exhibit their characteristic property of engulfing foreign particles from the circulation. 2. Non-toxic doses produce no histopathological changes in the tissues examined and the amount of thorium in the reticulo-endothelial cells is diminished after one month and greatly so after 6 months. 3. Toxic doses produce, in some instances, emboli and minute necrosis in the spleen and retrograde changes in liver and kidney which may or may not be permanent.

6214

A Study of Splenic Contraction in Various Animals.

E. v. HAAM, C. J. TRIPOLI AND E. B. LEHMAN.

From the Departments of Pathology and Radiology, Charity Hospital, and Louisiana State University Medical Center, New Orleans, La.

Since Barcroft's[1] work, a great number of authors have emphasized the importance of the spleen as a reservoir for red blood cells. Numerous experiments have been performed to visualize the dilatation and contraction of the spleen. Barcroft tried to observe the movements of this organ in the dog through a celluloid window which had been implanted in the upper left abdominal quadrant. Others outlined the spleen with small metal clamps and observed the changes in position of the clamps caused by the dilatation and contraction of the organ, by means of the fluoroscopic screen and roentgenograms. Benhamou, Viallet and Marchioni,[2] using special soft tissue technique, tried to visualize the spleen by taking radiograms with the subject in numerous and varied positions. We have not been able to use this method to our satisfaction.

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[1] Barcroft, *Erg. Physiol.*, 1926, **26**, 818.

The spleens of dogs, rabbits, guinea pigs and rats were roentgenographically visualized by means of intravenous administration of colloidal solutions of thorium dioxide (Thorotrast), and the contraction of the spleens studied under the fluoroscope and on radiographs after the injection of adrenalin, after bleeding and after shock. Hematocrit determinations of the peripheral blood were taken while the spleen was dilated, following intravenous administration of chloralose solution and at intervals of one minute, 3 minutes, 5 minutes, 15 minutes, and 30 minutes following intravenous injection of adrenalin. Simultaneously with the hematocrit, radiographs were made.

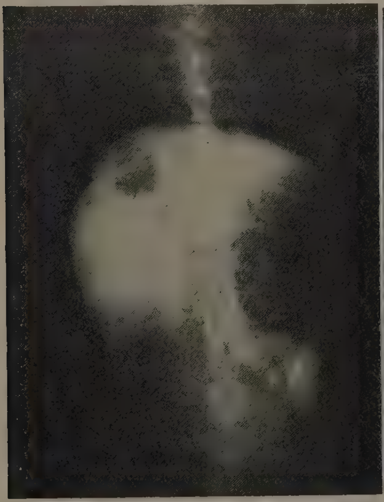

Fig. 1.

Fig. 2.

Fig. 1 shows the dilated spleen of a dog and Fig. 2 shows the contraction of the same dog's spleen 3 minutes after the intravenous injection of adrenalin. Hematocrit values of the peripheral blood cells showed an increase of 23% three minutes after the contraction of the spleen. Repeated experiments on the same dogs and on other dogs showed a correlation between the contraction of the spleen and the increase in the peripheral hematocrit reading. In the experiments with guinea pigs and rabbits, only slight, if any contraction of the spleen after adrenalin injection, bleeding or shock was produced. In accordance with this fact, erythrocyte counts and hematocrit determinations from the peripheral blood showed no notable increase after the administration of adrenalin. The spleen of rats showed distinct contraction after adrenalin, although the contraction is not as marked as in the dogs. These results are in accord with the experiments of Lauda and Haam,[3] who found that the spleen of rats is able to express only 3.3% of the total hemoglobin of the animal.

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[2] Benhamou, Viallet and Marchioni, *J. de radiol. et d'electrol.*, 1931, **15**, 147.

The important facts shown in our series of radiographs of splenic contraction in various animals are as follows: 1. Excellent contractions are obtained in dogs. The increase in the peripheral hematocrit determination is dependent upon the degree of contraction of the spleen. 2. The spleen of a rabbit and guinea pig has slight, if any, contractility powers. 3. The spleen of the albino rat shows contractions which are distinctly visible, but not as marked as the spleen of the dog. 4. Different results obtained in investigations concerning the variation of hematocrit blood volume in different animals can be explained by the fact that a different degree of contractility exists in the spleens of dogs, guinea pigs, rabbits, and rats.

6215

Observations on Decerebration by Embolism.

W. T. DAWSON AND OSCAR BODANSKY.

From the Department of Pharmacology, University of Texas School of Medicine.

Langley,[1] Mellanby,[2] and Edkins and Murray[3] have used intracarotid injection of starch as a method of decerebration for cats or rabbits. Observations in this laboratory on more than 40 dogs "decerebrated" with starch suspension (10%, 0.5 cc. per kg. or 2 cc.

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[3] Lauda, E., and Haam, E., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 260.

[1] Langley, J. N., *J. Physiol.*, 1912, **45**, 239.

[2] Mellanby, J., *J. Physiol.*, 1919, **53**, 1.

[3] Edkins, Nora, and Murray, Margaret M., *J. Physiol.*, 1924, **59**, 271.

total; opposite carotid tied before injection) showed irregular results. While superficial resemblance to decerebrates as regards muscle tone pattern, etc., was not infrequent, the method appeared unreliable, a result naturally to be expected in view of the inevitably large róle played by chance in the ultimate distribution of the embolizing particles. About one in 4 of the animals ceased breathing after injection.

Lycopodium spores, comparatively much less variable in size, more stable chemically[4] and on the average larger, are particles more readily identifiable in microscopic sections. The spore is a 3-sided pyramid with convex base.[5] Four spores are formed from a sphere of radius 21 microns.[4] There are about 95,000 spores per mg. against about 10,000,000 for rice starch.[4] Inclusion in lycopodium suspension of lampblack,[6] red oxide of iron, or the less permanent soluble dyes, methylene blue, crystal violet or diazine green, has enabled us to locate with moderate accuracy in gross sections the distribution of embolic material in the brain, so that its irregularity is plain, and the functional variation from animal to animal explicable. Attempts to explain functional results on an anatomical basis have proved interesting. Thus in an embolized dog, still breathing, but with relaxed limbs, the irregular posterior distribution of the embolic mixture extended to the pons-medulla junction and might conceivably have cut off the blood supply of the nuclei of Deiters. In the non-breathing the posterior limit was as a rule in the medulla or even the cord, in the few moving animals not even as far as the mid-brain.

The animals used for lycopodium embolism were: 35 dogs, 1 pig, 2 calves, 13 rabbits, 14 cats, 1 rhesus monkey.

We concluded that experimental cerebral embolism (1) as a method for decerebration, is as yet unreliable, (2) is useful for demonstrating by use of small groups of animals such simple relations as that embolism of the bulb may affect the persistence of respiration or central cardiac inhibition (failure of veratrum response), etc. We have found the entire operation is quite practicable under local anesthesia.

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[4] Wallis, T. E., *Pharmaceut. J. and Pharmacist,* 1919, **103**, 75.

[5] United States Pharmacopoeia, X.

[6] Dawson, W. T., *Am. J. Physiol.,* 1930, **93**, 635.

6216

Effect of Theelin on Immature Guinea Pigs.

E. L. KING AND ARTHUR G. KING.

From the Department of Obstetrics, Tulane University Medical School, and Charity Hospital, New Orleans, La.

It was previously pointed out that the vaginal smears of rabbits were not reliable as an index of hormonal activity, confirming the work of Kunde and Proud.[1] A more careful search of the literature reveals that Courrier[2] had previously observed that there was no histological change in the vaginal mucosa of several rabbits either during heat or after the administration of an ovarian extract.

We also reported an observation in 3 immature guinea pigs of the opening of the vulval orifice 48 hours after injection with the urine of a pregnant woman. To Courrier[3] again belongs the credit of having made the same observation in a single guinea pig that he injected with an ovarian extract. Laqueur[4] in studying the uterine hypertrophy of rodents after injection with an ovarian hormone, also noted the vulval opening in 2 guinea pigs but observed that one of his controls also had a wide open vagina at the end of the experimental period, namely a week. In trying to repeat our original phenomenon we found that it is not constant, appearing in but 2 of 23 animals injected with theelin-bearing fluids, while in 2 out of 34 guinea pigs the opening of the vulva, without, however, typical oestrus smears, appeared spontaneously.

Our attention was shifted, therefore, from the vulva to the effect of theelin on the uterus. Hypertrophy of the genital tract in immature animals in general is described as characteristic of the follicle hormone. Mice, rats, rabbits, and dogs, have been extensively investigated. But other than the two authors referred to above and Frank,[5] little seems to have been published. Jares[6] showed that guinea pigs are incapable of giving the Aschheim-Zondek reaction, although Papanicolaou[7] has recently demonstrated microscopic changes in the follicle following the injection into immature guinea pigs of the urine of pregnant cows.

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[1] King, E. L., and King, A. G., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 469.

[2] Courrier, R., *Comptes rendus Soc. de Biol.*, 1926, **94**, 280.

[3] Courrier, R., *Comptes rendus Soc. de Biol.*, 1924, **90**, 453.

[4] Laqueur, E., *Deutsch. med. Wchschr.*, 1926, **52**, 1247.

[5] Frank, R. T., Female Sex Hormone, 1929, Thomas Co., Baltimore.

[6] Jares, J. J., *Anat. Rec.*, 1931, **49**, 185.

[7] Papanicolaou, G. N., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 807.

Most authors have used the obvious hypertrophy of the uterus of rodents contrasted with a littermate control to demonstrate the uterine effect of the follicle hormone. Siddall[8] showed that in immature mice, animals weighing between 18 and 20 gm., the uterus (with the ovaries, tubes, and the cervix) weighed less than 1/400 of the total weight of the animal. Using the blood serum of pregnant women, he demonstrated the presence of theelin by daily injections over 5 days. At the end of that period the uterus was found to weigh more than 1/400 of the total weight, as for example, 1/300. Using the denominator as an index, a value over 400 would be negative, whereas a value under 400 would indicate uterine hypertrophy.

Although Frank states that in the immature guinea pig 4 to 6 days are necessary for hypertrophy of the uterus to take place, we have observed it regularly in 48 hours, and even 24 hours after the injection of theelin-bearing fluids. We have established a "ratio" between the weight of the uterus in mg. and the weight of the animal in gm. The normal should be 2.5 or less, corresponding, of course, to Siddall's value of 400. The advantages of this ratio are that it rises in value as the uterus increases in size whereas the Siddall number decreases in value. Again, the Siddall number implies an accuracy that is very hard to obtain, a change in the last significant figure representing 1/8 mg., while in the ratio as proposed an error of 25 to 36 mg. will just alter the last figure. Finally, the results in the immature guinea pig can be read in 48 or even 24 hours instead of 5 days.

The urine of pregnant women near term was used as a cheap and available supply of theelin, and 15 immature guinea pigs were injected from 7 different samples. The injections were so grouped that certain variables could be studied, although of course the number of cases is small. It was found that the intraperitoneal route gave slightly better results than the subcutaneous; that storage over night on ice did not affect the result; that the number of doses, distribution, and the amount injected had no effect on the outcome. This corresponds to the observations of others that the various reactions of the sex hormones are of a threshold type. On the other hand, one animal receiving 4.0 cc. in one dose died of peritonitis, and 3 others died of causes undetermined in 24 to 48 hours, without, however, interfering with the uterine hypertrophy. The weight of the animals seemed unimportant, although those guinea pigs in the neighborhood of 250 gm. gave the best results. An interesting observation was that 2 animals at least 6 months old but still sexually immature and weighing 350 gm., which had been injected with pregnancy urine some 3 months previously, gave typical expected reactions. In no case was any ovarian change visible to the naked eye observable. Finally the length of time between the injections and the sacrifice of the animal did not seem to play any great part in the degree of hypertrophy. The best results were obtained at the end of 6 days with one sample, but there was no progressive correlation, and in general 48 hours was ample time for the reaction.

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[8] Siddall, A. E., *J. Am. Med. Assn.*, 1928, **91**, 779.

The results with pregnancy urine were as follows:

Urine	Guinea Pigs	Ratio	Average
A	3	5.6-8.7	7.1
B	6	2.9-3.9	3.5
C	2	3.0-3.6	3.3
D			3.6
E			3.4
F			5.2
G			4.5

Since the urine of pregnant women contains large quantities of anterior pituitary-like hormone, to prove that the uterine hypertrophy was not due to this substance, 3 animals were injected with Antuitrin-S (Parke-Davis) in doses of 20, 40, and 120 units. The ratios were respectively: 2.1, 2.5, and 2.5, that is, all negative. On the other hand when Theelin (Parke-Davis) was injected in doses of 9, 35, and 90 units, the results were: 2.6, 3.5, and 3.0. This last experiment indicates again the threshold nature of the reaction, and also, that while 9 units will produce a minimal hypertrophy, 35 will give a definite reaction but 90 will not give any greater reaction. We are not ready yet, however, to discuss the quantitative relationships involved.

Among the controls, one 214 gm. guinea pig, receiving no urine, had a ratio of 1.9. Four samples of male urine were used to control the findings. This was unfortunate inasmuch as theelin has been reported on many occasions in male urine as well as in male feces, bile, and even blood, although usually in very small amounts. The results were as follows: Urine Z, 2.3 in each of 2 animals; urine Y, from the same individual some 2 weeks later, 4.0 and 4.3; urine X, 2.7; and urine W, 2.2. While standard tests for the demonstration of theelin in the male urines giving a positive reaction should have been made, we feel that it was the presence of this substance that was responsible.

Summary. Attention is called to the immature guinea pig as a suitable animal for demonstrating the presence of theelin by uterine

hypertrophy observable within 48 hours. There is proposed a numerical evaluation of this hypertrophy in the form of the ratio of the weight of the uterus in mg. to the weight of the animal in gm. Using this ratio the presence of theelin was demonstrated in pregnancy urine and a commercial preparation of theelin, differentiating these from a commercial preparation of the anterior pituitary-like hormone.

6217

Attempted Induction of Labor by Injections of Theelin.

J. THORNWELL WITHERSPOON.* (Introduced by E. C. Faust.)

From the Department of Obstetrics, Tulane University School of Medicine.

Setting aside as irrelevant to this discussion the effects which the hormones of the anterior hypophysis are supposed to exert upon the hormones of the ovary, we may accept as reasonably well established at least 3 facts: that the endometrial changes which precede menstruation are controlled during the first half of the menstrual cycle by the follicular hormone of the ovary; that they are controlled during the second half of the cycle by the lutein hormone of the ovary; and that the actual menstrual flow is precipitated by the withdrawal of the lutein hormone, with which is associated the beginning retrogression of the lutein body. To emphasize the positive rather than the negative aspect of the theory, we may say that since biologic inactivity is unlikely at any time, menstrual bleeding is initiated by the ascendancy of the ovarian follicular hormone over the lutein factor. Arguing from this premise, it does not seem unreasonable to assume that the onset of labor in a normal pregnancy which has reached term may be the result of a similar sequence of events, the ascendancy of the same follicular hormone over the same lutein hormone, which is known to be present throughout pregnancy.

To test this theory and in the hope of precipitating the onset of labor, a series of experiments was undertaken on 8 pregnant negro women at full term by injecting (subcutaneously) varying amounts

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* My thanks are due to Dr. E. L. King, Professor of Obstetrics in the School of Medicine of Tulane University, on whose service in Charity Hospital the experiments were conducted, and to Parke Davis and Company, who kindly supplied the Theelin used.

of theelin, the commercial preparation of the ovarian follicular hormone. At the same time, on the assumption that the follicular hormone can be secured by the aspiration of ovarian follicular cysts at laparotomy, fluid obtained from this source was injected into 2 other pregnant negro women who were also considered to be at term.

TABLE I.

Primipara or Multipara	Amt. Theelin Injected cc. per dose	Time elapsed between injections and onset of labor pains	Time elapsed between the injections and delivery
		hr.	hr.
M.	1	24	60
P.	2	24	40
M.	3 Injection painful	120	125
M.	4 Injection painful	Discharged not delivered	after 72 hr.
P.	½ 3 doses		”
M.	½ 3 doses		”
M.	½ 3 doses		”
P.	1 2 doses		”
P.	1†		96 hr.
P.	4†		80 hr.

† Ovarian follicular fluid used in place of Theelin.

The preceding table furnishes the details of the experiment. It is questionable whether in any instance the onset of labor followed the injection of the hormone soon enough to be considered the result of it. The labors were normal in every respect, as were the children born of them, and the only conclusion to be deduced from this very small series of cases is that the ovarian follicular hormone, when administered subcutaneously and in the described amounts, has no influence upon the onset of labor. Whether the results may be different if larger amounts were given or other modes of injection resorted to is a matter of speculation.

The questionable value of negative results, especially in a small series of cases, is fully appreciated, but since scientific and clinical data are built up negatively as well as positively, the experiment is published as a matter of record.

6218

Relationship of Pancreatic Digestion to the Emptying of the Gallbladder.

DANIEL N. SILVERMAN. (Introduced by E. C. Faust.)

From the Department of Medicine, School of Medicine, Tulane University.

This communication reports experiments made as a continuation of previous efforts to determine the mechanism of the stimulation of the gallbladder by ingested egg yolk. Boyden[1] first showed that a mixture of egg yolk and cream caused a rapid expulsion of bile from the animal gallbladder, later that in the human subject the administration of egg yolk and cream, but not lean meat or carbohydrate, caused a rapid expulsion of bile.[2] Boyden[3] believed that the gallbladder musculature does not respond to food in the gastrointestinal tract unless digestion and absorption have occurred. The late Professor Denis and the writer showed, by analyzing blood taken at intervals after administration of fats, that emptying of the visualized gallbladder did not depend on the factor of absorption. We then found[4, 5] that the already digested egg yolk (fatty acids and glycerol) did not affect the gallbladders which were emptied by plain egg yolk. This suggested some relationship between gallbladder emptying and the intestinal digestion of fat. With the advice and assistance of Professor A. C. Ivy of Northwestern University, experiments were undertaken to determine the relationship of pancreatic digestion to the evacuation of the gallbladder.

Exp. 1. A male dog was laparotomized, the 2 pancreatic ducts were ligated near their entrance into the duodenum, the contents of the gallbladder were aspirated completely and through the same needle sufficient quantity of brominized oil was injected to distend the gallbladder. The abdomen was closed. The animal was kept in the fasting state and 12 hours later, a radiograph was made. As a control another plate was taken 2 hours later. Then, 4 yolks of raw eggs were ingested by the animal. The emptying of the gallbladder is shown (Fig. 1).

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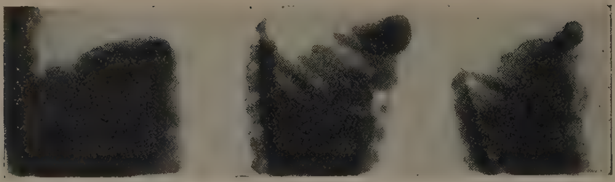
[1] Boyden, E. A., Abstracted in *Anat. Rec.*, 1923, **24**, 388.

[2] Boyden, E. A., *Anat. Rec.*, 1925, **30**, 333.

[3] Boyden, E. A., *Anat. Rec.*, 1926, **33**, 201.

[4] Silverman, Daniel N., Denis, W., On the Relationship of Gallbladder Emptying to Ingested Fats, Radiology.

[5] Silverman, Daniel N., Denis, W., and Weinberger, Herbert L., *Am. J. Med. Sci.*, 1929, **177**, 3.

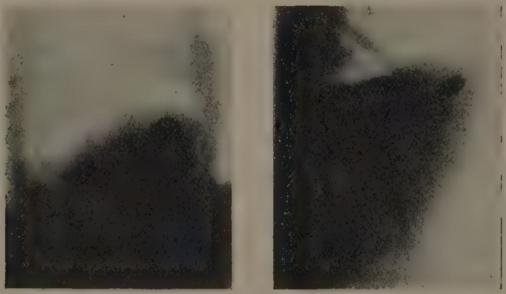


Fasting Controls After Fat

FIG. 1.

Demonstrating Emptying of Gallbladder Following Ingestion of Egg Yolk. (Pancreatic Ducts Ligated.)

Exp. 2. Two weeks later the same dog used in Exp. 1 was given tetraiodophenolphthalein by mouth. After a 16-hour fast with the gallbladder visualized on the radiograph, 4 yolks of raw eggs were administered. A definite emptying of the gallbladder was noted (Fig. 2)



Fasting After Fat

FIG. 2.

Demonstrating Emptying of Gallbladder Following Ingestion of Egg Yolk. (Pancreatic Ducts Ligated.)

Three days following the second experiment the dog was laparotomized by Dr. Bliss and myself and inspection of the pancreatic ducts showed them both to be stenosed.

Summary. The experiments indicate that pancreatic digestion is not essential to the mechanism of gallbladder evacuation following the ingestion of fat.

Appreciation is extended to Drs. Leon Menville and G. N. Ané for the valuable assistance rendered by them in the radiographic work.

6219

Heat Regulation in Medullary and Mid-brain Preparations.

A. D. KELLER AND W. K. HARE.

From the Department of Physiology and Pharmacology, University of Alabama School of Medicine.

One of us approached the localization of the central mechanism for the control of body temperature by attempting to determine if the fibre tracts concerned could be delimited as they passed caudally through the brain stem.[1,]* It was soon noted that a medullary preparation differed markedly from a mid-brain preparation in its ability to heat regulate. The absence of heat regulation in a mid-brain preparation has long been known; this fact was demonstrated particularly well by Bazett and Penfield[2] in chronic mid-brain and pontile cats. Bazett[3] more recently emphasized that he has never been able to elicit shivering in such a preparation, yet Dworkin[4] reports shivering in acute medullary rabbits.

Our observations have been derived from some 30 mid-brain preparations and 10 or more medullary animals, of which 4 only were maintained more than 2 days postoperative. The mid-brain preparations exhibited the following reactions: (a) shivering was never observed even when the rectal temperature was lowered in the ice box to 26°C.; (b) it was always necessary to keep the animals in a heated incubator in order to maintain a relatively normal temperature[5]; (c) hyperthermia never resulted from central trauma, meningitis, or from other infections such as peritonitis and consolidation of the lungs; (d) sweating was never observed to result, even when the rectal temperature was raised to 43-44°C.; (e) typical panting has been observed only in a very few instances and in these cases the sections were high, but respiratory rates of 120 were frequently obtained on over-heating.

On the other hand, medullary preparations responded as follows: (a) shivering was readily elicited and frequently occurred in the acute stage as a result of heating or after injecting hot saline subcu-

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[1] Keller, A. D., *Am. J. Physiol.*, 1930, **93**, 665.

* The final observations in this particular problem are being withheld from publication until the present study has been extended further.

[2] Bazett, H. C., and Penfield, W. G., *Brain*, 1922, **45**, 185.

[3] Bazett, H. C., *Abstracts Am. Physiol. Soc.*, 1932.

[4] Dworkin, S., *Am. J. Physiol.*, 1930, **93**, 227.

[5] Keller, A. D., *Am. J. Physiol.*, 1932, **100**, 576.

taneously, as well as from cooling or injecting cold saline subcutaneously; (b) although the preparations were housed in a heated incubator directly following operation, within a few days they maintained an adequate rectal temperature in unheated cages; (c) hyperthermia was observed in 2 instances at ordinary room temperature where the lesion was highly traumatic, with sub-stem hemorrhage; (d) heating the animals resulted in a progressive slowing of respiration and finally periodic breathing, of the nature that occurs immediately after making the brain lesion[6]; (e) sweating has not been observed, however, young animals have not been used. No cases of infections have been seen.

The difference between 2 such preparations, *e. g.*, in their ability to maintain a rectal temperature of relatively normal range when subjected to a low environmental temperature, was readily demonstrated by preparing the animals simultaneously and then placing them in the same incubator. In this case if the box was regulated to maintain the temperature of the mid-brain animal, the medullary preparation soon died of hyperthermia; on the other hand, if the box was regulated for the medullary preparation the temperature of the mid-brain animal was markedly low. This contrast is shown in the following table:

TABLE I.

	Cat 103—Medullary		Cat 104—Midbrain	
Day	Rect. Temp. °C.	Box Temp. °F.	Rect. Temp. °C.	Box Temp. °F.
5	35	82	31	85
6	38	80	32	82
7	37.7	74*	32.5	82
8	41	80*	Died from tracheal obstruction	
8:45 a. m.	Icebox	50		
10:30	37.7	Typical generalized shivering..		
12 noon	36	Cat taken out. Still shivering		
2:00 p. m.	38.7	78* No shivering		

* Animal in unheated cage.

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[6] Keller, A. D., *Am. J. Physiol.*, 1931.

6220

The Hypothalamus and Heat Regulation,

A. D. KELLER AND W. K. HARE.

From the Department of Physiology and Pharmacology, University of Alabama School of Medicine.

Isenschmid and Schnitzler[1] localized the chief central mechanism for the control of body temperature in the hypothalamus by demonstrating that a hypothalamic rabbit heat regulated, whereas, a mid-brain preparation did not. Would extirpation of the hypothalamus alone without injury to other parts of the brain-stem render the animal incapable of heat regulating? In a few isolated cases in high mid-brain preparations, typical panting and vaso-dilation was readily elicited and persisted at relatively low rectal temperatures[2]; however, subsequent preparations were made in which this response could not be obtained under any condition. It therefore seemed probable to us that the "heat regulating center" was really in the cephalic mid-brain and was consistently spared functionally only by a transection passed ahead of the hypothalamus and rarely by a section passing nearer to it caudally. By the lateral approach (elevation of the temporal pole) the hypothalamus has been successfully sectioned free from its cephalic, dorsal, and caudal connections with the brain stem with no resultant hemorrhage, and without disturbing the blood supply to adjacent brain tissue.

The hypothalamus was sectioned free from its connection on one side (unilateral removal) without disturbing bilateral heat regulation so far as could be determined grossly. An incomplete section which crossed the mid-line to involve the opposite half of the stem, impaired the power of the animal to maintain body temperature. Thus a cat with approximately a three-quarter section maintained a rectal temperature of 36°C. housed in an unheated cage. However, when put in the ice box its rectal temperature fell slowly in spite of the presence of shivering. This condition supervened 3 months postoperative in the same state as the first week after operation. The animal panted typically and vigorously when heated.

When the whole of the hypothalamus was sectioned free (or macerated) with the caudal level of the section ranging through the middle level of the mammillary bodies or slightly ahead, the ability to maintain a constant body temperature was completely lost and shivering could not be elicited. Such an animal exhibits a remarkable release from control of the heat loss mechanism. Continuous polypnea and cutaneous vasodilation supervened. The respiratory rate varied with the activity of the animal, decreasing below 100 as a rule during sleep and ranging around 150 per minute (without the mouth being open) after activity. A mere pressing of the tail elicited typical panting (mouth open and tongue out) with a rate over 180. Such preparations have been maintained for 2 weeks without any change in this picture, suggesting that we are probably dealing with a true release phenomenon, since the picture supervenes as soon as the animal recovers from anesthesia.

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[1] Isenschmid, R., and Schnitzler, W., *Arch. f. Exp. Path. u. Pharm.*, 1914, **76**, 202.

[2] Keller, A. D., *Am. J. Physiol.*, 1930, **93**, 665.

These observations indicate that the chief central mechanism controlling heat production is located in the hypothalamus, and that extirpation of this region releases the heat loss mechanism, located elsewhere, from coordinated control.

6221

Effect of Carbon Arc Irradiation on the Goitre Producing Substance in Plants.*

BRUCE WEBSTER. (Introduced by J. H. Musser.)
(With the technical assistance of Catherine Bender.)

From the Department of Medicine, Tulane University School of Medicine.

The occurrence of a seasonal and annual variation in the goitre-producing power of cabbage and other plants has been previously reported.[1] At that time it was suggested that sunlight might be a factor in bringing about the synthesis of some substance in the cabbage responsible for the production of goitre. Freshly harvested cabbage, from a source which had previously been known to produce active goitrogenic plants, was finely divided, spread in layers from 1 to 2 cm. thick, and irradiated with a carbon arc for 20 minutes at a distance of 50 cm. The maximum intensity of the arc used was between 3500 and 4000 A. U. The cabbage was then fed to normal rabbits in quantities equivalent to 60 calories per kilo per day. The experiment was controlled by feeding hashed, unirra-

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* Aided by a grant from the Committee on Grants-in-Aid, National Research Council.

[1] Webster, B., Marine, D., and Cipra, A., *J. Exp. Med.*, 1931, **53**, 81.

diated cabbage from the same source in equivalent amounts to a similar series of rabbits. All rabbits used in the experiment were injected subcutaneously with 5 mg. of potassium iodide one week prior to the beginning of the experiment, to insure complete involution of their thyroid glands. At the termination of the experiment, the condition of the thyroid gland was determined during life by its exposure under ether anesthesia. The animals were later sacrificed and the weight of the thyroid was taken at autopsy.

Table I shows the results.

TABLE I.
Rabbits fed 67 days.

Rabbbit No.	Material Fed	Condition Thyroid at Termination (Operation)	Wt. Thyroid at Autopsy* mg.
22	Irradiated hashed cabbage	+++	575
23	" " "	++++	620
24	" " "	+++	480
27	" " "	+++	540
25	Plain hashed cabbage	++	320
26	" " "	+—	180
28	" " "	++	280
29	" " "	+	210

* The weight of the normal thyroid gland in our series of rabbits varies between 50 and 150 mg.

A second experiment was carried out under the same conditions except that a batch of cabbage with less goitre producing activity was used. The thyroid glands were exposed at operation but the animals were not later sacrificed. Table II shows the results of this experiment.

TABLE II.
Rabbits fed 96 days.

Rabbit No.	Material Fed	Condition of Thyroid at Termination (Operation)
45	Irradiated hashed cabbage	+++
46	" " "	+++
47	" " "	++
42	Plain hashed cabbage	+ ?
43	" " "	++
44	" " "	+ ?

It would appear that carbon arc irradiation increases the goitre producing power of cabbage. It would seem that light brings about a photochemical reaction in the plant with the consequent formation of the goitrogenic substance. This agrees with the suggestion of

Marine and his associates[2] that the goitre producing power of cabbage was due to its cyanogen content. Further experiments are in progress.

6222

Effect on Kidney Function of Ether, Ethylene, Ethylene and Amytal, and Ethylene and Avertin.

ROBERT P. WALTON. (Introduced by J. T. Halsey.)

From the Department of Pharmacology, School of Medicine, Tulane University.

Although the suppression of kidney function by anesthetics has been studied under various conditions there is yet considerable speculation involved in their selection when considered on this basis. Marx[1] has shown, in dogs, that higher dosages of amytal exert a strong anti-diuretic effect and cause retention of substances ordinarily excreted through the kidneys. The clinical observations of Emge and Hoffmann[2] indicate a suppression of diuresis for the 2 post-operative days following amytal. Bruger, Bourne and Dreyer[3] consider that kidney function is more depressed with avertin than with amytal. Kugel[4] and Bonsmann[5] have reported marked variations in the anti-diuretic effect of various hypnotics.

The work reported here is an attempt to compare the anti-diuretic influence of some common anesthetics under conditions which may have a bearing on their clinical usefulness. Normal dogs were maintained for several days on a regulated intake of food and water while the total 24 hour excretion of urine, urea, chloride and phosphate was ascertained. The excretion of phenolsulphonephthalein (1 cc. of 0.6% solution intramuscularly) and water (20 cc. per kg. by stomach tube) was determined each morning over a 2 hour period. The volumes of urine secreted in the afternoon and night were also noted. (For convenience in catheterizing, female dogs were used after making a posterior vaginal slit.) Approximately constant amounts of food and water, given in the late afternoon were so adjusted that the dogs consumed the full diet almost immediately. At about the middle of each period of days, anesthesia was instituted for one hour immediately following the morning administration of water and phenolsulphonephthalein. Satisfactory surgical anesthesia, with abolition of leg and abdominal muscle tone, was obtained with sodium isoamyl-ethyl barbiturate (32 to 40 mg. per kg. intraperitoneally) and tribromethyl alcohol (400-500 mg. per kg. rectally) when supplemented by ethylene-oxygene mixtures of 90-10 ratio or of less ethylene concentration. Ether was administered by cone and a comparable depth of anesthesia was maintained. Ethylene-oxygene mixtures alone, serving as something of a control both for excitement and ethylene effects, were administered for an hour in one series and, with the ratio used (85-15), no anesthesia was obtained. In all experiments on which conclusions are based the animals were sufficiently recovered by late afternoon to consume their regular diet of food and water.

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[2] Marine, D., Baumann, E., Spence, A., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 772.

[1] Marx, *J. Pharm. Exp. Therap.*, 1931, **41**, 483.

[2] Emge and Hoffman, *Am. J. Surg.*, 1930, **9**, 16.

[3] Bruger, Bourne and Dreyer, *Am. J. Surg.*, 1930, **9**, 82.

[4] Kugel, *Arch. Exp. Path. Pharm.*, 1922, **142**, 166.

[5] Bonsmann, *Arch. Exp. Path. Pharm.*, 1930, **156**, 160.

The results of 3 to 6 experiments with each anesthetic combination indicated clearly that the 24-hour output of urine was not substantially diminished by such anesthesias, either on the day of administration or the few days immediately following. The average decrease noted (10 to 15% on the day of anesthesia) was not considered a significant diminution in that the normal fluctuations were of the same order. Likewise, the 24 hour secretion of urea and of chloride was not significantly affected. In some cases, the 24 hour phosphate output was definitely augmented by the administration of ether, as has been reported by Stehle and Bourne,[6] but no definite effect on phosphate excretion was noted with the other anesthetics. The 2 hour water diuresis response was almost totally inhibited by each type of anesthesia and, correspondingly, the urine output was considerably increased in the afternoon period immediately following. This recovery of diuretic function was not as prompt with amytal as with the other anesthetics used. The average of the 2 hour phenolsulphonephthalein excretions was depressed by anesthesia (to about 40% of the total dye injected) but on the subsequent day was manifested to the normal degree (about 70%), no marked variations being noted among the different anesthetics.

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[6] Stehle and Bourne, *J. Biol. Chem.*, 1924, **60**, 17.

6223

Effects of Some Basal Anesthetics on Vasomotor Reflexes.

C. F. LACEY. (Introduced by J. T. Halsey.)

From the Department of Pharmacology, School of Medicine, Tulane University.

In a study of the relative efficiency of basal anesthetics it was frequently noticed that in animals under varying doses of barbiturates (amytal, allonal and dial) the blood pressure showed a depressor response upon stimulation of the central end of the divided sciatic nerve. As such reversal of normal reflexes has been noted with other drugs such as chloroform, it seemed worth while to investigate the effect produced on vasomotor responses by these barbiturates and those produced by some other basal anesthetics or hypnotics.

Cats were used, and satisfactory experiments were conducted with the different basal anesthetics in the following numbers (amytal 4, dial 6, and allonal 5). Under the influence of novocain, a tracheal cannula was inserted in order to administer ethylene 90% oxygen 10% for anesthesia to complete the preparation, that is to cut the sciatic and insert a carotid cannula to record the blood pressure.

The central end of the sciatic was stimulated with a Dubois Raymond coil connected with 3 dry cell batteries in parallel to give a voltage of 6. As the response of different cats to the same strength of stimulus varies so greatly no average figures for the stimulation used can be given. The minimal strength required to produce a vasomotor response after 20% of the M.L.D. of these drugs was stimulation with the secondary coil at 20 cm. in some cats, while in others no response would be obtained until the secondary coil was at positions intermediate between this and 11 cm.

The barbiturates were given intraperitoneally. The usual procedure was to give 20% of M.L.D.* while the cats were still under the ethylene, and after a fixed interval (10 minutes was sufficient for the full effect to be produced) the ethylene was discontinued and 5 minutes allowed for its effect to be lost. Then the sciatic was stimulated with gradually increasing strengths of the current, beginning in each period with a stimulus which produced no vasomotor response. Subsequently, additional doses of 10% of the M.L.D. were administered and the same procedure repeated until from 60 to 70% of the M.L.D. had been given. In the majority of instances, the first vasomotor response noted in cats under the influence of larger doses of the barbiturates (30% or more of M.L.D.) was a fall in blood pressure, at times of only about 10 mm. of mercury, while at others the fall amounted to as much as 20 mm. With the largest doses (from 60 to 70% of M.L.D.) the strongest stimulation which could be applied frequently produced no vasomotor response. However, with these large doses, the cats were deeply anesthetized, and the blood pressure was already low as compared with the preliminary blood pressure, the average for the preliminary blood pressure being 152 mm., while the average under the above mentioned dosages of the barbiturates was 100 mm.

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* M.L.D. for amytal and dial 100 mg. per kilo, for allonal 90 mg.

The most common vasomotor responses under doses from 30 to 60% of the M.L.D. with weak currents was a fall in blood pressure, and the same fall was also often the result of stronger stimulation. However, it was a common experience that, in these experiments, on increasing the strength of the stimulus by moving the secondary coil one or more centimeters the depressor response was changed to a pressor one. At times the fall in blood pressure appeared to be due to vagal inhibition of the heart. Consequently, a number of experiments were carried out with vagotomized cats. In these animals, the vasomotor responses to sciatic stimulation were similar to those of the cats with the vagi intact except that the fall in blood pressure in many cases was much less. In no instance was the fall as much as 20 mm. of mercury.

With corresponding percentages of the M.L.D. of chloral, avertin and paraldehyde† (the first 2 given rectally, the last intraperitoneally) comparative observations were made using the same procedure as with the barbiturates.

In animals under these drugs, with the vagi intact the usual responses with currents that would produce any effect, was an increase in the blood pressure. Sometimes, however, a fall was noted, which appeared to be due to vagal inhibition of the heart. In vagotomized cats under the influence of these drugs, sciatic stimulation invariably caused a rise in blood pressure, except that with the largest doses (above 60% of M.L.D.) the strongest stimulation possible with the coil used was often followed by neither vasomotor nor other reflexes.

This difference in the effect of these different basal anesthetics on the vasomotor mechanism would appear to be of significance. Freeman and Rosenbleuth[1] report that dial has no unusual effects on the vasomotor reflexes. A repetition of their observations with currents of the strength and with the dosages used by them has failed to confirm this statement, in that we have found variable vasomotor responses, either pressor or depressor to sciatic stimulation.

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† M.L.D. chloral 350 mg. per kilo, for avertin 400 mg., for paraldehyde 2.5 cc.

[1] Freeman and Rosenbleuth, *Am. J. Physiol.*, 1931, **98**, 454.

6224

Attempts to Produce Hyperplasia in Thyroid by Destruction of Part of the Adrenals and by Infection.

HOWARD R. MAHORNER. (Introduced by A. Ochsner.)

From the Department of Surgery, Tulane University School of Medicine.

Several methods may produce retrogression in the hyperplastic thyroid, *viz.*, resection of the thyroid, administration of iodine, x-ray, but it is more difficult to produce the hyperplasia characteristic of exophthalmic goiter at will.

The purpose of these experiments was to note the microscopic changes occurring in the thyroid after injury to and destruction of part of the adrenals or in the organism suffering from an infection. In these experiments dogs were used. Dogs in this locality apparently very rarely have goiter. The thyroid of a 6 to 10 kg. dog is usually about 2.2 x 1.2 cm. in size.

To have a section of thyroid to compare the result by, from each of the dogs a piece of thyroid about 2 x 2 mm. was removed at a preliminary operation. Sections removed from the same dog at subsequent operations or at necropsy were compared with this first section. Sections removed subsequently from control dogs in whom no other procedure but this preliminary operation was done did not show any hyperplasia, as has been proven to occur when larger pieces of the thyroid gland are removed.

Two dogs were fed 3 grains of thyroid extract a day for 46 and 47 days, respectively. These dogs became extremely restless, nervous, excitable, and had even a stare but no exophthalmos. Sections removed from the thyroids of these dogs after feeding the animals thyroid extract for 46 and 47 days showed retrogressive changes in the thyroid; smaller epithelial cells with more colloid in the vesicles. This response to thyroid extract has been described before.[1]

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[1] Mosser, W. B., *Am. J. Surg.*, 1929, **7**, 338.

Cole and Womack[2] found that infection in dogs resulted in hyperplasia of the thyroid gland. I implanted a small coil of wire contaminated by a pure culture of staphylococci into the sternocleidomastoid muscle of dogs. The dogs were sacrificed after 4, 9, 17, and 24 days. The thyroid in 2 showed hyperplasia but the thyroid of the other 2 showed no change. Moreover, thyroid glands removed from 2 other dogs that died of pneumonia showed hyperplasia. One of these had part of the adrenals destroyed. A dog dying of peritonitis following intestinal obstruction showed no change in the microscopic appearance of the thyroid. Another dog having a periadrenal abscess showed no hyperplasia. Thus, 50% of 8 dogs with infections showed hyperplasia in the thyroid.

Golyakowski[3] and Marine and Baumann[4] found that ligating the vessels and removal or injury of the cortex of the adrenal glands was followed by an increase in the metabolic rate in a certain percentage of animals. After preliminary operation to obtain a section of the thyroid for comparison at a second operation, I crushed one or both adrenals, macerating them with an Ochsner forceps. I attempted to destroy a large portion of the glands and yet not both glands entirely. I can report subsequent examinations of the thyroids of 10 animals at various periods from 5 to 38 days. Seven of these showed no change, one showed retrogression, and 2 showed slight hyperplasia. One of these dogs showing hyperplasia in the thyroid died of pneumonia; thus, infection may have played a part.

The thyroid was measured at the first and at subsequent examinations in some of these dogs. No gross changes were noted in size or appearance of the gland.

The dogs in whom the adrenals had been injured moved slowly, seemed pepless, became thin and emaciated, disinterested, and looked just the opposite from the dogs that had thyroid extract.

The changes in these thyroids are very meager indeed. Whenever there was an apparent decrease in the amount of colloid and heaping up of epithelial cells, I called it hyperplasia. It is certainly not the striking change found in thyroids in exophthalmic goiter in the human. These changes are in reality only suggestive; they are not constant. As Webster,[5] McCarrison[6] and others have shown, goiter may be produced by goiterogenic diet, but I am not aware of a typical exophthalmic goiter having ever been produced or ever existed of itself in the lower animals. There is something else in man, the emotional side or some other factor, that makes goiter sometimes different from the goiter occurring in animals.

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[2] Cole, W. H., and Womack, N. A., *J. Am. Med. Assn.*, 1929, **92**, 453.

[3] Golyakowski, quoted by Marine and Baumann (Ref. 4).

[4] Marine, D., and Baumann, E. J., *Am. J. Physiol.*, 1921, **57**, 135.

[5] Webster, B., Marine, D., and Cipra, A., *J. Exp. Med.*, 1931, **58**, 81.

[6] McCarrison, R., *Brit. Med. J.*, 1929, **1**, 5.

6225

Peritoneal Lavage. Effective Elimination of Nitrogenous Wastes in the Absence of Kidney Function.

SIDNEY BLISS, ARTHUR O. KASTLER AND S. B. NADLER.

From the Department of Biochemistry, Tulane School of Medicine.

The experiments of one of us have demonstrated that nephrectomized dogs eliminate considerable amounts of ammonia in vomiting. These results have been amply confirmed and extended by others. Martin has studied the elimination of ammonia, urea and other nitrogenous wastes lost in this manner.

During a similar study on elimination of both urea and ammonia in the vomitus, we have demonstrated that really considerable quantities of urea and ammonia are thus eliminated. However, the prospect of this method of elimination serving as a therapeutic measure to tide patients over temporary periods of deficiency in kidney function is not bright. Elimination by way of the stomach becomes quantitatively important only when nitrogen retention has reached a very serious stage, and in our hands this mode of excretion has always failed to keep pace with nitrogen accumulation.

During the course of these experiments we encountered edema of massive proportions. The ascitic fluid in these cases was found to be rich in urea and practically in equilibrium with the concentration of that substance in blood.

We have been able to accomplish the elimination of nitrogen wastes by the introduction of a solution of balanced salts into the peritoneal cavity of a nephrectomized dog, allowing time (10 minutes) for diffusion of nitrogenous wastes into this fluid, and then drawing off the fluid through the trocar which remains in position throughout. We introduce on the average 750 cc. of solution, allow it to remain in the peritoneal cavity for 10 minutes and then allow it to flow out by gravity. Varying numbers of such washings are made at a single insertion of the trocar. Depending upon the severity of the retention at the time, we have done as few as 5 and as many as 20 washings at one sitting. The relief of nitrogen retention is prompt and dramatic. The plasma value has been reduced from 160 mg. to 80 mg. per 100 cc. within 3 hours. The survival period of untreated dogs is about 3 days, while with peritoneal lavage we have had survival periods of 13 and 16 days.

The therapeutic possibilities are at present under investigation here. Our studies on kidney-functionless dogs have included one dog anuric with uranium nephritis, one dog with one kidney removed and the other ureter ligated, and 5 doubly nephrectomized dogs.

TABLE I.

Control of Plasma Non-protein Nitrogen by Peritoneal Lavage.

Dog No.	Plasma Non-prot. N.		Non-protein N. of Lavage Fluid		Remarks
	Before lavage	After lavage	Conc.	Amt.	
	mg. %	mg. %	mg. %	gm.	
2	156	141	101	4.0	double nephrectomy
3	140	103	68	5.4	
	107	94	60	4.9	
	159	95	68	6.8	
	212	110	31	3.2	
	182	79	70	6.2	double nephrectomy
	144	96	70	5.8	
	135	120	48	2.1	
	182	80	67	8.4	
	142	80	58	5.5	
	104	57	44	3.7	
	106	56	29	5.6	
4	76	54	19	1.8	
5	46	45	13	1.6	
	133	68	36	3.1	
	162	102	48	4.1	
	138	62	62	6.9	double nephrectomy
	190	75	75	6.6	
	120	82	44	4.0	
6	191	110	62	5.2	
	267	148	65	5.1	anuric: uranium nephritis
	230	143	72	6.7	
7	250	124	74	9.9	left kidney removed right ureter ligated
8	143	96	47	3.6	
	160	86	48	3.7	double nephrectomy

The washings from the peritoneal cavity have been examined for the presence of the ordinary urinary constituents. We have identified phosphates, creatinin, urochrome, urea, sulfates, chlorides (in excess of the amount in the wash fluid) and traces of protein.

Illinois Section.

Northwestern University Medical School, May 24, 1932.

6226

Pain: Time of Occurrence Following Temporary Coronary Occlusion.

DON C. SUTTON AND H. C. LUETH. (Introduced by A. C. Ivy.)

From Northwestern University Medical School.

Lewis,[1] by an ingenious method, studied the time factor in the production of pain following temporary complete obstruction of the brachial artery. After an interval of 70 to 75 seconds, obstruction of the brachial artery caused unbearable pain. He thinks that during this interval a change in metabolites may be the factor in producing pain and has called this the "P-factor". Lewis used as a criterion of pain, that unbearable end point in order to eliminate the psychic factor. In our earlier experiments when the coronary artery was temporarily occluded, pain was observed immediately.[2, 3] The question raised by Lewis as to the discrepancy apparent between the results by different methods of study suggested further investigation.

Method. 20 dogs were used. Briefly, the chest was opened under ether anesthesia, and artificial respiration given at once. The pericardium was exposed, opened, and a tough linen suture passed around the ramus descendens branch of the left coronary artery as close to its origin as was technically possible. This suture was passed through a flanged glass tube, a purse string suture held the tube firmly in place in the pericardium and prevented an open pneumothorax. The chest wall with the tube projecting was tightly sutured. After a period of not less than 4 hours following the completion of the operation, traction on the suture will occlude the vessel and produce a painful response. At the conclusion of all experiments the animals were sacrificed and the position of the ligature verified by postmortem examination.

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[1] Lewis, Sir Thomas, *Arch. Int. Med.*, 1932, **49**, 713.

[2] Sutton, Don C., and King, W. W., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 842.

[3] Sutton, Don C., and Lueth, H. C., *Arch. Int. Med.*, 1930, **45**, 827.

Results. The typical result is that of immediate pain response to slowing of blood flow during constriction. The time interval is too short to be easily accurately measured. There is a variability in different animals as to the degree of response dependent upon a number of factors. The time of complete recovery from anesthesia varies in different animals. In those animals in which 4 hours or more elapsed from the time of operation a well-defined pain response occurred immediately, or within 3 seconds following operation. When less time was allowed for recovery the pain response was delayed and not as clearly defined. There is an occasional animal in which the response to any form of pain is slight or delayed. Under these conditions the longest interval recorded was 14 seconds in one dog, in the remainder the interval was not longer than 3 seconds. In most cases repeated attempts decreased the time of response, so that after many trials they so closely followed obstruction that the exact interval could not be measured. One animal showed an instantaneous painful response followed by a period of unresponsiveness, which lasted several minutes, after which the pain could be provoked as promptly as ever. Careful autopsy examination failed to furnish any clue to explain this reaction.

This apparent discrepancy in the time required to produce pain by the method of Lewis and that employed by us appears to be mainly the question of the definition of pain. The dog probably responds to a much less degree of stimulation than that endured by an individual during the experiment of Lewis; in fact, this is evident on sustained coronary vessel compression. Secondly, when one considers the difference in degree of shock observed in coronary occlusion and occlusion of a peripheral artery, it is not improbable that the pain of cardiac muscle differs from that of voluntary muscle.

6227

The Beneficial Effect of Mucin and Chondroitin in Experimental Liver Damage.

L. A. CRANDALL, JR., G. M. ROBERTS AND J. W. GIBBS.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Kim and Ivy[1] have observed that mucin prevents ulcers and greatly reduces weight loss in biliary fistula dogs while alkali therapy also prevents peptic ulcer but does not moderate the impairment of nutrition. This, together with improvement in nutrition observed by those who have employed mucin in the therapy of human peptic ulcer, suggests that this substance, in addition to having a local action in the bowel, may play a rôle in metabolism. Of interest in this connection is the fact that many of the conditions in which chronic ulcer develops in the dog are associated with liver damage, biliary fistula, bile duct ligation, pancreatic duct ligation, Eck fistula, and cinchophen poisoning. It seemed worthwhile to study more specifically the effect of mucin on the nutritional state of animals with hepatic damage in which ulcer does not occur as frequently as in biliary fistula dogs.

A series of 9 dogs with ligation of the common bile duct were studied. Of these, 4 were kept as untreated controls and 5 were given 60 gm. of mucin once daily. In addition, 2 Eck fistula dogs that had lost weight post-operatively were given the same amount of mucin daily. All were kept on the stock diet of corn-meal made to a mash with bone soup and bread, and with a small amount of horse-meat added.

This series is too small to allow of definite deductions. However, in view of our previous experience with such animals, the results are so striking that it is felt a preliminary report is warranted. The control animals ran the usual course, losing 30, 33, 26, 35%, average 31%, of their weight in 30 days, while the mucin-fed dogs lost 11, 7, 7, 2, 10, or an average of 7.3%, in the same time. The increases in weight of the Eck fistula dogs fed mucin are given in Table I.

This effect of mucin could be due to its local action in increasing absorption from the gastro-intestinal tract. It seemed more likely, however, that it is due to some constituent of the mucin. Since glucuronic acid is contained in the mucoitin fraction of the mucin molecule and since glucuronic acid is used by the liver in detoxifi-

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[1] Kim, M. S., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 686.

TABLE I.
Eck Fistula Dogs on Mucin and Chondroitin Feeding.

	Mucin		Chondroitin	
	No. 1 kg.	No. 2 kg.	No. 3 kg.	No. 4 kg.
Preop.	17.7	11.4	15	22.4
Before mucin	15.4	8.6	11.4	18.1
After mucin				
Days				
14	15.6	8.7		19.2
18			12.6	19.5
30			13.6	
44	18.4	9.7		
80	19.1	10.5		
110		11.3		

cation, we attempted to prepare mucoitin from mucin. This procedure was unsatisfactory since the yield is small. Chondroitin sulphuric acid, however, was prepared from cartilage in large amounts by modification of the method of Levene which avoided the use of barium carbonate, since we found it impossible to remove the last traces of barium when the material was prepared by the original method.

Chondroitin sulphuric acid was fed to 2 Eck fistula dogs in amounts of 1 gm. per day and resulted in weight increases as given in Table I. The usual course of the Eck fistula dogs, as observed in more than 60 animals in this laboratory by one of us (L.A.C.) in the last few years, is a progressive post-operative loss of weight which may proceed to death or may stabilize when the animal has lost from 10 to 40% of its original weight. The animals used in the present experiment were either maintaining their weight at considerably below the pre-operative level or were still losing. A spontaneous gain in weight in such animals is extremely rare.

In addition to the weight gains marked changes in the appetite and activity were observed and the dogs fed with mucin or chondroitin sulphuric acid became more vigorous and ate much better than did the controls.

6228

Observations on a Physiologically Active Substance Appearing During Anaphylactic Shock.

ERICH GEBAUER-FUELNEGG, CARL A. DRAGSTEDT AND RALPH B. MULLENIX.

From the Department of Research Bacteriology and the Department of Physiology and Pharmacology, Northwestern University Medical School.

We have previously reported[1] observations on the appearance of a physiologically active substance in the thoracic duct lymph and the inferior vena cava blood of dogs during anaphylactic shock. It was reported that this substance had the property of contracting the smooth muscle of the guinea pig intestine, and that this contraction was observed equally well in the intestine of guinea pigs immunized to the antigen used in sensitizing the dogs. In this respect the nature of the active substance appears to be somewhat different from the "hepatic anaphylatoxin" of Manwaring and his coworkers.[2]

Preliminary observations on the nature of the active substance occurring in the thoracic duct lymph and the inferior vena cava blood show it to have the following characteristics. The active substance is dialyzable, indicating that it has a small molecular size. Using a 3-compartment electro-dialyzer the active substance concentrates in the basic compartment showing it to be of basic nature. The intensity of physiological activity and the Pauly reaction are parallel and the physiological activity is neutralized after condensation with diazotized sulphanilic acid. This indicates that the activity is associated with an imidazole compound. Pharmacological observations with the active substance indicate that it depresses the blood pressure of an etherized, atropinized cat; and while contracting the smooth muscle of the guinea pig intestine has no effect on the intestine of the mouse. In this respect it resembles histamine.[3] A characteristic wheal is produced in human skin by endermal inoculation of an active dialysate. Additional chemical and pharmacological investigations are in progress, but it may be stated that at the present time we have made no observations which are incompatible with the assumption that the active substance may be histamine.

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[1] Dragstedt, C. A., and Gebauer-Fuelnegg, E., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 891.

[2] Manwaring, W. H., Marino, T. C., McCleave, T. C., and Boone, T. H., *J. Immunol.*, 1927, **13**, 319.

[3] Wedum, A. G., and Gebauer-Fuelnegg, E., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 888.

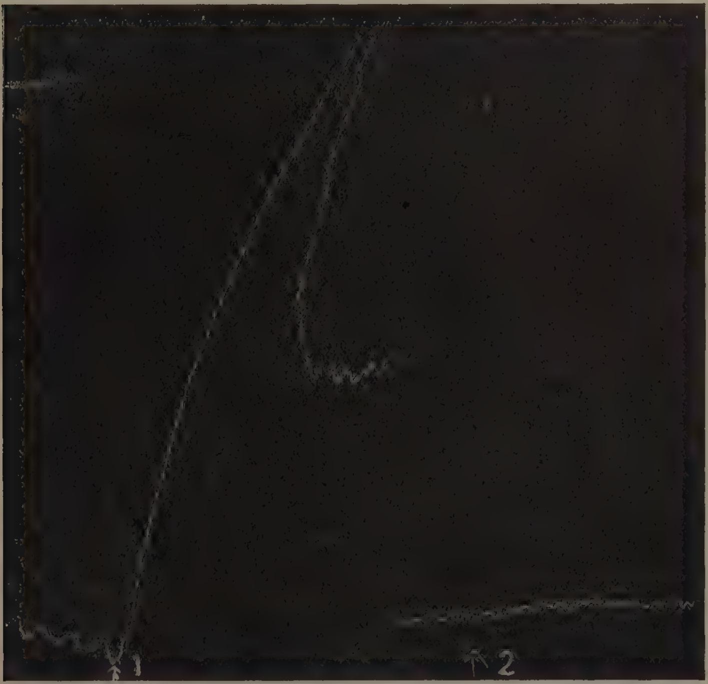
6229

Chemical Inactivation of Histamine.

E. GEBAUER-FUELNEGG. (Introduced by C. A. Dragstedt.)

From the Department of Research Bacteriology, Northwestern University Medical School.

The inactivation of histamine can be accomplished by a variety of means. Some of its physiological actions are antagonized by a proper dosage of adrenalin or related compounds. A specific oxidative enzyme—histaminase—is found in certain organs capable of destroying the base.[1] The addition of formaldehyde to surviving smooth muscle preparations previously contracted by histamine was shown to relax the muscle. As an explanation the formation of a



1 = .003 mg. Histamine-2 HCl. 2 = 1.0 mg. Histamine + 2 mols. Pauly.
FIG. 1.

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[1] Best, C. H., and McHenry, E. W., *J. Physiol.*, 1930, **70**, 349.

physiologically inactive histamine-formaldehyde complex was postulated.[2] The following observation is an addition to the above list of inactivation methods.

It was found, that if to histamine solutions of known concentration are added 2 mols. of diazotized sulfanilic acid in the presence of Na_2CO_3 the resulting dye (Pauly's reaction) will not contract the surviving intestinal strip of the guinea pig if the solution is brought to a physiological pH. On the other hand an insufficient molar quantity of the diazonium compound will cause only a partial inactivation of the base. This observation was found to be useful when the presence of histamine is suspected in biological extracts, etc. Because of the possibility that the tyramine-diazotized sulfanilic acid condensation product would also result in an inactivation of the tyramine or an analogous effect might be observed if smooth muscle contractive protein split products were treated as above, this test should be taken only as additional evidence for the presence or absence of histamine.

6230

Gelatin as an Opsonizing Substance.

W. J. NUNGESTER AND SAMUEL LIPSTEIN.

From the Department of Bacteriology and Patten Research Laboratories, Northwestern University Medical School.

Since the introduction of the term opsonin by Wright and Douglas the conception of the mechanism of action of opsonins has rather constantly tended to point toward a deposition of such substances on the surface of the particle to be phagocytized. Recent studies are well exemplified by the experiments of Mudd, Lucké, McCutcheon and Strumia,[1] who have shown that the globulin fraction of immune sera contains a substance or substances which will unite with the antigen, bacterial cells or inert colloidal particles coated with a protein, in such a fashion that surface properties of the antigen as cohesiveness and cataphoresis are altered in association with such immunological reactions as agglutination and increased phagocytosis.

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[2] Kendall, A. I., *J. Infect. Dis.*, 1927, **40**, 689; see also ref. 1.

[1] Mudd, Lucké, McCutcheon and Strumia, *J. Exp. Med.*, 1929, **49**, 779; 1930, **52**, 313.

If phagocytosis is stimulated by such a coating on the surface of the antigen, as Mudd and his coworkers postulate, it might be possible to use substances other than serum or its various protein fractions to so prepare the surface of the antigen for phagocytosis. Since the completion of our work Freund[2] has published his experimental studies in which he used tannin and observed phagocytosis of red blood cells in the absence of serum. He was thus able to repeat the earlier experiments of Reiner and Koppz.[3]

Since gelatin has long been used to coat particles of one sort or another with a view of altering their surface physics we began our experiments using this substance. We employed a commercial bacteriological sheet gelatin made up in saline to the designated concentration and adjusted to pH 7.4. The results were also confirmed with a second gelatin furnished by Dr. Gebauer-Fuelnegg and prepared from the skin of hogs by mild chemical extraction.

In our first experiments we made use of the Wright capillary pipette method estimating the percent of polymorphonuclear cells showing phagocytosis, after 15 minutes incubation at 37°C. This method was soon abandoned because it was noted that the blood cells in the presence of gelatin tended to settle out on standing. A method was made use of in which the reacting substances were pipetted into small paraffined tubes which were subjected to gentle mechanical agitation in a water bath for 15 minutes after which smears were made and examined in the usual fashion. Our first experiments were done with a suspension of living staphylococci but we later used a killed suspension of *Streptococcus scarlatinae* as described by Jung and Day.[4]

TABLE I.
Varying Concentrations of Normal Serum and Gelatin as Opsonizing Agents.

% Concentration of Serum	100	50	25	12.5	6.2		
% Phagocytosis	35	24	17	16	16		
% Concentration of Gelatin	10	5	4	3	2	1	0.5
% Phagocytosis	36	30	25	26	32	19	8

Saline control—4% Phagocytosis.

The table shows the effect of varying concentrations of gelatin or human serum on the phagocytosis of the killed streptococcus suspension. In experiments of this type 0.05 cc. of the opsonin (serum or gelatin dilution) or saline in the control, 0.05 cc. of bacterial suspension and 0.10 cc. of washed human blood cells were the quantities employed. These results indicate that gelatin can serve as an opsonin increasing the phagocytosis from 4% in the saline control to 36% in the case of 10% gelatin. Although in this particular experiment the gelatin curve happens to indicate a slightly greater phagocytosis with the concentrations of gelatin used than with the serum, in many of our experiments, when undiluted serum and gelatin in 2% or 3% concentrations were compared, the phagocytosis in the serum usually ran from 30% to 40% while the gelatin results ranged from 20% to 30%. The saline controls rarely showed more than 5% phagocytosis.

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[2] Freund, J., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 876.

[3] Reiner and Koppz, *Z. f. Immunitäts.*, 1929, **61**, 397.

[4] Jung and Day, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1080.

We have tried a limited number of other substances with a view of explaining the mechanism of the opsonic action of gelatin.

Fresh white of egg in concentrations of 12% or less has failed to give any evidence of aiding phagocytosis in the absence of serum. Even in concentrations of 25% to 50% the egg white yielded negative results except on one occasion when 16% phagocytosis was observed with a 1-1 dilution. Since the relative viscosity of a 25% egg white solution is greater than that of a 2% gelatin solution, viscosity may not be the important factor in explaining the action.

The buffer values of nutrient broth, 1% gelatin and saline, were compared and found to decrease in that order. Since phagocytosis takes place in the presence of 1% gelatin and is absent in the presence of the more highly buffered nutrient broth, it is possible that the buffering value of the gelatin is not sufficient to explain its action as an opsonic substance.

Attempts have been made to wash bacterial cells sensitized with gelatin but the results indicate that the gelatin is easily removed by washing. Hektoen[5] has shown that in immune serum as well as in normal serum the heat stabile opsonic substance is firmly held to the bacterial or blood cell surface despite washing and, although alone has little effect on phagocytosis, with a small amount of normal serum greatly increases phagocytosis. We were unable to demonstrate that gelatin possessed any analogous property.

Conclusion. We have shown that washed human polymorphonuclear leukocytes will phagocytize staphylococci or streptococci in the presence of gelatin without the addition of serum.

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[5] Hektoen, *J. Inf. Dis.*, 1909, **6**, 66.

6231

Postural Reactions in Cats Following Destruction of Both Red Nuclei.

W. R. INGRAM AND S. W. RANSON.

From the Institute of Neurology, Northwestern University Medical School.

In the course of experiments to throw light upon the relation of the red nucleus to postural reactions, the Horsley-Clarke stereotaxic instrument was used for the stimulation as well as destruction of this structure in cats. No postural responses could be obtained from the red nucleus upon direct stimulation, although the areas of the reticular formation dorsal and lateral to it gave reactions of a coordinated type affecting limbs and trunk.[1] Destruction of the nucleus bilaterally by electrolysis, with a minimum of damage to tissues elsewhere in the brain stem, produced in both acute and chronic experiments preparations which were able to right themselves, stand, and walk in an efficient, if somewhat dysmetric, manner. The righting mechanism did not depend upon visual reflexes, as shown by experiments in which these were eliminated by enucleation. The gait of the animals was marked by some asynergia, probably traceable to involvement of the *brachium conjunctivum* and hence the cerebellum. There was also a certain amount of stiffness, and in many cases the balance of tonus was noticeably in favor of the extensor muscles. Under suitable conditions, as when the animal was suspended in a hammock, or supine, slight increase of extensor tonus could be definitely shown by the presence of the positive stutz reaction or resistance to passive flexion in each limb. This extensor hypertonus was not of sufficiently high grade to interfere with the normal activities of the animal. In general, these experiments may be said to indicate that while the red nucleus plays an important rôle in the regulation of postural tonus, it is but a part of a more complex system devoted to this important function.

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[1] Ingram, Ranson and Hannett, *J. Neurol. and Psychopath.*, 1932, **12**, 219.

6232

Neutralization of Poliomyelitis Virus by the Serum of Liberian Negroes.

N. PAUL HUDSON AND EDWIN H. LENNETTE.

From the Department of Hygiene and Bacteriology, University of Chicago.

Epidemiological studies of acute anterior poliomyelitis in the United States have suggested that the virus of this disease is more widespread than is indicated by the incidence of clinical cases (Frost,[1] Aycock[2]). Aycock and Kramer[3] interpreted the high proportion of serums from "normal" persons of Georgia that neutralized the virus, as meaning that a large number of persons in the Southern United States are exposed to the virus, thereby acquiring an immunity with a low attack rate. They concluded that the extent of the distribution of the virus in warmer climates is equal to that in cooler climates. Soule and McKinley[4] have reported that of 8 Porto Ricans without history of attack or exposure to this disease and whose serums were examined for their neutralizing property, all gave positive results.

The understanding of the epidemiology of poliomyelitis is assisted by studies in the geographical distribution of the disease. It occurred to us that we might get data by examining the serums of "normal" natives of West Africa for neutralizing properties. We obtained specimens through the courtesy of Dr. J. B. Rice of the Firestone Plantations of Liberia and Dr. W. A. Sawyer of the International Health Division of the Rockefeller Foundation. The technic is that which has been standardized for similar purposes in this laboratory, with the use of the virulent Rhoads' PMV strain of virus. One part of 1.25% suspension of monkey cord is mixed with 3 parts of serum; the mixture is incubated 2 hours at 37°C. and held in the refrigerator over night. Two cc. of the mixture is injected intracerebrally into *Macacus rhesus*. Temperatures and observations are recorded daily for 6 weeks.

Ten specimens have been examined. Eight of the test animals showed no symptoms and survived. A ninth monkey and the convalescent serum control monkey had a facial paralysis and survived without other symptoms or a febrile rise. The tenth animal and the virus control died of experimental poliomyelitis.

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[1] Frost, W. H., *Hyg. Lab. Bull.*, No. 90, 1913, Washington, D. C.

[2] Aycock, W. L., *J. Prev. Med.*, 1929, **3**, 245.

[3] Aycock, W. L., and Kramer, S. D., *J. Prev. Med.*, 1930, **4**, 201.

[4] Soule, M. H., and McKinley, E. B., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 168.

The age distribution of serum samples is: two 15 years, one 16, one 18, two 20, and one each of 23, 25, 26, and 35 years. The person furnishing the negative specimen was 25 years of age. An inquiry into the incidence of this disease in Liberia disclosed a lack of recorded data on the subject. Rice[5] writes that he has not seen or heard of poliomyelitis cases but points out that his practice is confined to adult employees; he adds that the infrequent paralyses seen might be interpreted as following this condition. Strong and Shattuck[6] have encountered several cases of probable poliomyelitis in Liberia. Johnson,[7] Director of the Medical and Sanitary Service of Nigeria, British West Africa, informs us that acute cases of poliomyelitis are occasionally observed in the natives of that region.

The significance of the neutralization test in this connection implies that it is a specific immune reaction, which has yet to be established.

6233

Effect of Pancreatic Juice on Absorptive Mechanism of the Gall Bladder.

EDMUND ANDREWS, M. GOFF AND L. HRDINA.*

From the Department of Surgery, University of Chicago.

Wolfer[1] showed that pancreatic juice injected into the gall bladder of the dog caused marked histologic changes in the wall of the viscus. The possibility of this being the cause of cholecystitis clinically was thoroughly discussed by him. The secretory pressure of the pancreas is much higher than that of the liver[2] and in case of blockage of the ampulla by stone, anatomic arrangements are such in a high percentage of human subjects that the gall bladder might fill with pancreatic secretions.[3, 4]

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[5] Rice, J. B., personal communication.

[6] Strong, R. P., and Shattuck, G. C., Harvard African Exped., 1926-27. Harvard Univ. Press, Cambridge, 1930, **1**, 360.

[7] Johnson, W. B., personal communication.

* The work was done in part under a grant from the Rosenwald Foundation.

[1] Wolfer, J. A., *Surg., Gyn. and Obstet.*, 1931, **53**, 433.

[2] Dragstedt, L. R., personal communication.

[3] Cameron, A. L., and Noble, J. F., *J. Am. Med. Assn.*, 1924, **87**, 1410.

[4] Westphal, K., *Z. f. klin. Med.*, 1929, **109**, 55.

Our studies on 19 dogs were aimed to determine if the presence of pancreatic juice would affect the permeability of the gall bladder to the constituents of the bile, especially the bile acids. The pancreatic juice (kindly furnished by Dr. L. R. Dragstedt) was activated and contained about 1000 units. It was injected directly into the gall bladder by needle puncture in some cases, in others, through a ureteral catheter up the common and cystic duct. Four cc. were used. The duration of the experiments was 4 days. The results were as follows:

1. Pathological changes of mild degree were evident in the gall bladder wall in most cases. These, however, were not marked. The susceptibility of the dog's gall bladder to slight stasis and infective influences, as we have previously noted[5] is so marked that it would be hazardous to assert that the changes found were not due to the manipulations. 2. In some experiments the juice was Berkefeld filtered. In these experiments the pathological and chemical changes were much less than from the unfiltered juice. 3. The average cholesterol content of the bile was not reduced, but the concentration of bile acids was reduced to less than one-third of the values found in control experiments. This is in addition to the fact that the total fluid contents of the gall bladders was considerably less than normal. 4. In 5 experiments with unfiltered juice all the bile salts had been absorbed, leaving the cholesterol precipitated. 5. In *in vitro* experiments with mixtures of bile and pancreatic juice, no such changes were found indicating that in the *in vivo* experiments the bile salts were absorbed and not destroyed.

Conclusion. The presence of pancreatic juice in the dog's gall bladder causes a marked differential absorption of bile salts at the expense of cholesterol, and hence tends to precipitate the latter.

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[5] Andrews, E., and Hrdina, L., *Arch. Surg.*, 1931, **23**, 201.

6234

Action of Human "Pernicious Anemia Liver Extract."

O. RICHTER,* A. C. IVY AND M. S. KIM.†

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

How the antianemic principle in liver which is active in pernicious anemia produces its effect is not known. Neither is it known whether the active principle is stored and depleted, like certain vitamins, nor if the liver *in vivo* secretes it in a hormone-like manner for the purpose of governing hematopoiesis. It was considered desirable to ascertain whether the liver of a pernicious anemia patient contains the antianemic principle. Since an opportunity to perform 2 experiments bearing on this question occurs so rarely at present, we desire to record the results we have obtained. The livers of 2 pernicious anemia patients were extracted. The first extract was made from a liver of a pernicious anemia patient who had received liver extract prior to her death sufficient to produce a reticulocytosis, the bone marrow at autopsy showing an active hemopoietic response. The liver was extracted and the extract administered intravenously to 3 pernicious anemia patients with positive results. The second extract was made from the liver of a case of pernicious anemia who entered the hospital in a moribund condition and died shortly after entrance. Because of her rapid exitus, the liver extract she received intramuscularly and intravenously did not produce an active hemopoiesis, which was shown by the absence of reticulocytosis and hemopoietic activity in the bone marrow. An extract was made from this liver and administered to a pernicious anemia patient subcutaneously with no result.

Experiment I. A white female patient, 75 years old, entered the hospital in a semi-stuporous state. Pernicious anemia had been diagnosed 5 years previously. The classical findings with achlorhydria and spinal cord complications were present. The patient had received therapy in a haphazard manner during the past 5 years. During the past month she had been confined to bed but had received no therapy for a period of months. In addition to the presence of pernicious anemia, evidences of cystitis, nephritis, and myocarditis were present at this time. The urine was 3 plus for albumin and contained many leucocytes. The blood urea was 45.5;

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* Chapell Foundation Fellow.

† Eli Lilly Fellow.

creatinine, 2.8; blood sugar, 111; red blood count, 750,000; hemoglobin, 27; white blood cells, 3,600; a blood film characteristic of pernicious anemia. The patient had a septic temperature and her condition became progressively worse. Liver extract for parenteral injection not being available, the patient was started on liver extract by mouth on September 19th, and was given 1600 cc. of normal saline and 1000 cc. of glucose solution hypodermically. The patient was given an average of 3½ oz. of potent liver extract in small divided dosages daily, receiving a total of 42 oz. during the 12 days of the treatment, each ounce equivalent to 240 gm. of whole liver. Because of her poor condition, she was also given 400 cc. of whole blood intravenously. The day of the transfusion, or on the seventh day of liver therapy, the blood examination revealed R.B.C., 1.67; Hb.,41; W.B.C., 9,100; reticulocytes, 25.4%. The patient rallied for several days and appeared to be improved but died 7 days after the transfusion from the complications. An immediate autopsy showed generalized severe anemia (pernicious type); combined degeneration of spinal cord; pseudo-membranous cystitis; septic tumor of spleen; parenchymatous degeneration of liver, kidneys, and myocardium; severe coronary sclerosis; bone marrow showed evidence of hyperactive erythropoiesis with similar embryonic cells in splenic meshes.

The liver was immediately extracted by a slightly modified method for preparing fraction G (Minot) for intravenous use. Eight hundred grams of liver were available for extraction. Animal tests for toxicity of the prepared extract showed it to be non-toxic for dogs. Thirty cc. of the final extract was equivalent to 100 gm. of liver.

Three patients with pernicious anemia were given the extract intravenously to determine whether the active "anti-pernicious anemia" principle is present in the liver of the pernicious anemia patient. The 3 patients receiving the extract responded. After the response was obtained, the patients were placed on liver extract (Chapell, Rockford, Illinois) by mouth, a typical liver therapy remission resulting.

Case 1. Typical case of pernicious anemia. A young woman with first relapse of six weeks' duration. Results recorded in Table I show a marked reticulocyte rise following 4 daily injections of human pernicious anemia liver extract, a total of 14 cc. intravenously.

Case 2. An old case of pernicious anemia with several previous relapses, who failed to react to liver by mouth. A woman 68 years of age. Results in table show a reticulocyte response following

TABLE I.
Response of 3 Pernicious Anemia Patients to Intravenous Administration of Extract of Liver of a Pernicious Anemia Patient.
Case on Liver Therapy Sufficient to Cause Reticulocytosis.

Patient No.								Days					
		1	2	3	4	5	6	7	8	9	10	11	12
1	Dose cc.	3.5	3.5	3.5	3.5								
	Hb %	17				20					23		
	RBC	0.89				0.83					1.39		
	WBC	3.6				2.8					4.0		
	Reticulocytes %	0.2	1.4	1.4	1.6	2.4	3.8	7.4	16.4	27.4	32.6		
2*	Dose cc.		3.0										
	Hb. %	29			41					44			
	RBC	0.89			1.08					1.49			
	WBC	4.7			4.3					3.6			
	Reticulocytes %	1.4			0	8.4	5.0	4.2		0.4			
3	Dose cc.		4.0	3.5	3.5		4.0		3.5				
	Hb. %	53					57						69
	RBC	1.8					1.86						2.74
	WBC	6.0					5.4						5.6
	Reticulocytes %	0	0	0	0	0.8	2.8	2.6	1.8	1.4	2.4	2.4	0.4

* Failed to react to liver extract by mouth.

only one intravenous injection (3 cc.) of human pernicious anemia liver extract.

Case 3. Pernicious anemia with a somewhat higher initial Hb. and R.B.C. count in male, 63 years of age, associated with a chronic myocarditis. Results recorded in table show no marked reticulocyte response but a marked increase in Hb. and R.B.C. in 11 days following intravenous injections of human pernicious anemia liver extract. Also followed by a marked clinical improvement. Patient received 5 intravenous injections, a total of 18½ cc.

The results, which are definitely positive, show that the active antianemic principle in pernicious anemia is present in the liver of the pernicious anemia patient recently treated with liver extract by mouth. The fact that one patient gave a response with 3.0 cc. of the extract (the equivalent of 10 gm. of liver) and the fact that Case 1 responded very well with 14 cc., leads us to believe that the active principle is present in approximately the same concentrations as in normal cattle liver.

Experiment II. A white female patient, 55 years of age, entered the hospital in a semi-stuporous state. The history obtained was that she had been ailing for the past 4 years. Tachycardia and paroxysmal attacks of dyspnea began 4 years ago following an illness which at that time was diagnosed as heart disease. Gastrointestinal disturbances were also experienced since then, consisting of occasional attacks of anorexia, and diarrhea. She was fairly comfortable and able to carry on her daily activities until 7 months ago, when she began feeling weak. The weakness became progressively worse so that in the past month she was confined to bed. Anorexia and vomiting occurred more frequently in the past months, associated with loss of appetite, strength and weight. She had never been on any form of liver or liver extract therapy throughout her entire illness.

The essential findings on entrance were an icteric tinge of the skin and mucous membranes, an atrophic tongue, a slightly enlarged heart, with a rapid, regular apex beat of 160 per minute, and a slightly enlarged liver and spleen. The blood and spinal Wassermanns were negative. The urine was negative. The blood urea was 32.69; creatinine, 2.05; blood sugar, 136; red blood count, 810,000; hemoglobin (Sahli), 29; white blood cells, 3,900; and a blood film characteristic of a primary anemia of the pernicious type. A suitable donor for a blood transfusion was unobtainable. She was started on parenteral liver extract, receiving a total of 7 cc. of which 4 cc. was given intramuscularly and 3 cc. intravenously. Her general condition became worse, the patient expiring 5 days after her entrance to the hospital. Daily reticulocyte counts were made, but no response was recorded. Autopsy showed severe generalized anemia (pernicious type); fatty changes and hemosiderosis of the liver; severe fatty degeneration of the myocardium; arterio- and arteriolosclerosis of the kidneys; atrophy of the gastric mucosa; chronic recurrent verrucous endocarditis of the mitral valve with insufficiency, hyperplasia of the bone marrow, but no hyperactive response as observed in the previous case.

The liver was extracted as in the previous case. From 2 to 4 cc. was injected daily for 9 days subcutaneously into a typical case of pernicious anemia. The reticulocyte count was taken daily and a complete count at 5 day intervals. The patient received a total of 31 cc. of liver extract (100 gm.) subcutaneously. Daily reticulocyte counts were taken, but no response was recorded in 13 days. The patient was then given one ounce of Chapell's liver extract t.i.d. by mouth which was followed by the usual hemopoietic response (Table II).

The results in this experiment show that the liver of this pernicious anemia patient who died before she had received effective liver therapy, did not contain the antianemic principle active in pernicious anemia.

The results of these 2 experiments indicate that the "specific" antianemic substance in liver is absent from the liver of the patient suffering from pernicious anemia and that when the antianemic

TABLE II.

Response of Typical Case of Pernicious Anemia Patient to Extract of Liver of a Pernicious Anemia Patient Who Received an Insufficient Amount of Liver Therapy to Produce Hemopoietic Response Because of Her Sudden Exitus.

Patient No.		Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
4	Dose cc.	2	4	0	4	4	4	4	4	5	*	*	*	*	*	*	*
	Hb. %	41					34			30					35		
	RBC	1.63					1.24			1.04					1.23		
	WBC	2.5					3.0			3.6					5.0		
	Reticulocytes %	0	0	0.8	0.6	1	0	0	0.2	0.4	0.4	0.4	0.2	1	3.4	13.9	27.8

* Failed to respond to this human liver extract so was started on 10th day with potent liver extract by mouth, one ounce t.i.d. showing typical reticulocyte response.

substance is administered to the pernicious anemia patient the liver stores it. This observation denotes that the active principle is similar in certain respects to the vitamins, particularly the "fat soluble" vitamins in regard to storage and depletion. Clear cut experimental evidence shows that Vitamins A, D, and E are stored and rather rapidly depleted, and also that B and G are stored and depleted, the evidence not being so clear cut in the case of the latter 2. Vitamin C is stored and depleted in the guinea pig, but in the rat either the rate of utilization of C is very slow, or it is synthesized or produced during digestion. However, the C content of skeletal muscle apparently disappears before a detectable change occurs in the liver. The fact that the antianemic principle in liver for pernicious anemia is present in other tissues, but in less concentration, is analogous to the distribution of vitamins, also that a definite latent period precedes the development of anemia when liver therapy is discontinued, is analogous to the latent period characteristic of vitamin deficiency.

On the basis of the observations of Castle,[1] which indicate that gastric digestion in normal man releases substances from meat that have an antianemic value in pernicious anemia, the most logical interpretation of our results is that the "active principle" of liver is not a fixed or integral part of liver protoplasm and may be stored and released physiologically by the liver *in vivo* to govern hematopoiesis.

6235

A New Disease of Moose. II.

G. I. WALLACE, LYELL J. THOMAS, ALVIN R. CAHN.
(Introduced by F. W. Tanner.)

From the Department of Bacteriology and the Zoological Laboratory, University of Illinois.

Thomas and Cahn[1] described a new disease of moose *(Alces americana americana)* in northern Minnesota. The symptoms of the disease were described, with the associated blood picture. Transplanting ticks *(Dermacentor albipictis)* from an infected moose to guinea pigs and rabbits produced a disease showing identical symptoms, with the same associated blood picture as in the moose. Profound cellular changes in the blood elements and the presence of bacteria were mentioned. The organism was isolated. The cellular changes will be discussed in the final report; the present paper deals with the isolated organism.

The first recognized bacteria-like organism was found in smears of the intestinal contents of ticks engorged with the blood of diseased moose. With a modified Wright stain these organisms showed a capsulated coccoid bacterium. This intestinal content was cultured on dextrose agar, and the organism isolated. A saline suspension of this pure culture was inoculated intravenously into guinea pigs and rabbits, and all of the experimental animals died within 28 hours, exhibiting symptoms similar to those shown by guinea pigs and rabbits infected through the medium of the tick itself. The organism has been repeatedly recovered from the liver, spleen, lungs, heart, kidney, brain, bone marrow and urinary bladder of inoculated and infected animals. This organism has also been inoculated into a bull, a chicken, a ram and a 4-day-old lamb. The chicken and the lamb died with symptoms exactly like those obtained in the guinea pigs and rabbits. In the case of the bull, 5 cc. of the culture was given intravenously. Within one hour after the injection very definite disturbances were noted, including a marked physical depression, listless drooping, labored respiration followed by lung hemorrhages, coughing and bloating. For 3 days the bull ran a high temperature, reaching 105.8°. Four days later the bull was normal to all outward appearances. However, blood smears taken 8 days after the inoculation, reveal a picture typical of the diseased moose and very similar to the blood picture of the infected guinea pigs and rabbits. Blood cultures made at this time yielded an abundant growth of the organism. The work on the ram and the bull is being continued.

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[1] Castle, *Am. J. Med. Sci.*, 1929, **178**, 748, 764.

[1] Thomas, L. J., and Cahn, A. R., *J. Parasit.*, 1932, **18**, 4.

The fact that some of the rabbits and guinea pigs died within an hour following the injection of the culture, together with the observations on the bull, lead the writers to suspect the action of a toxin. Consequently a 5-day-old broth culture was filtered and the sterile filtrate inoculated into a rabbit, a lamb and a chicken. The rabbit died within an hour, with symptoms identical with those shown in the rabbits receiving the culture injection. In the chicken the symptoms were identical with those shown by the chicken receiving the culture, for a period of about 2½ hours following the injection; then, however, the toxin chicken showed definite signs of recovery, and in 36 hours was apparently normal. The lamb acted in a manner comparable to that of the bull.

The culture was fed to guinea pigs, and on the 6th day after feeding, one animal showed a periodic paralysis of the hind legs.[1] Subcutaneous injections of the culture produced light symptoms similar to those produced in animals receiving intravenous injections. One pig received the injection along the mid-dorsal region of the back, directly over the spine, and showed more definite symptoms than the one which received the injection in the inguinal region. After 3 days the symptoms gradually diminished. The experiments involving subcutaneous injections and feeding are being enlarged and continued.

The organism has been repeatedly isolated from ticks taken from a diseased moose. It has also been isolated from guinea pigs which died after the tick *Dermacentor albipictis* from a diseased moose had engorged upon them. In every case in which death followed the injection of the culture, the organism has been reisolated. It grows very abundantly on all ordinary laboratory media. Its cultural reactions are now being studied. It may be stated that it is a vigorous fermentor, is capsulated, and that it apparently produces a toxin. The bacterium is a rod form with a tendency to assume a coccoid shape. The growth on agar is excessively mucoid; in broth it grows very well throughout the medium with a great deal of sedimentation. It produces Beta hemolysis on blood agar. Growth is exceedingly rapid, covering the entire surface of an agar slant within 5 hours. Indications are that it may be a member of the Klebsiella group.

6236

Chemical Changes in Infected Dogs' Gall Bladder Bile.*

L. E. DOSTAL AND EDMUND ANDREWS.

From the Department of Surgery, University of Chicago.

Previous experiments[1] have been reported showing that bile salts are absorbed faster than cholesterol from the infected dog's gall bladder. The importance of this lies in the fact that it is the bile salts that hold the cholesterol in solution and if the ratio falls below a certain critical level, cholesterol will be precipitated out of the bile. In former experiments the cystic ducts were closed so that no more bile could enter the gall bladder. In the experiments here reported the ducts were not ligated so that it was possible for bile continually to pour into the gall bladder and further concentration of cholesterol to occur, while the bile salts are progressively absorbed.

Streptococci and filtered emulsions of dog feces were used, the latter instead of pure cultures as they represent a better cross section of the intestinal flora. The injections were made by needle punc-

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* This work was done in part under a grant from the Douglas Smith Foundation for Medical Research.

[1] Andrews, E., Schoenheimer, R., and Hrdina, L., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 945.

ture and by insertion of a ureteral catheter up the common duct into the gall bladder. In the latter cases the common duct was ligated at the site of injection. The accompanying table shows that the bile salt content was reduced on the average to about 25% of normal figures while the cholesterol content was approximately doubled.

TABLE I. *Injection of Bacteria into Dog's Gall Bladder.*
Figures for bile salts and cholesterol in mg. per 100 cc. Duration of experiments, 4 days.

No. of Exps.		B.S.	Chol.	B.S./ Chol.
4	2 cc. cloudy suspension of streptococcus injected up common duct into gall bladder with catheter	1706	92	19
2	2 cc. same through needle puncture into lumen	1545	111	14
4	2 cc. filtered emulsion dog feces up duct	88	86	1
3	2 cc. same into lumen	217	118	2
2	2 cc. same subserous	1230	300	4
	Average	893	126	7
81	Normal controls	3630	55	66

In vitro control experiments show that no such changes are effected by the action of bacteria on bile.

That this rise in the cholesterol content is not due to secretion of cholesterol by the gall bladder wall is shown by the previously reported series of 57 experiments in which closed infected gall bladders showed no increase in the cholesterol content.

Conclusion. The infected gall bladder of the dog absorbs bile salts and continues to concentrate cholesterol to levels at which it can no longer be held in solution by the bile salts.

6237

Influence of Saponin on Absorption of Calcium Salts from the Intestine.

F. C. STERNASTY AND N. M. FELICELLI. (Introduced by T. E. Boyd.)

From the Department of Physiology and Pharmacology, Loyola University School of Medicine.

We have followed the curve of blood calcium concentration in normal dogs following the administration of calcium lactate in quantities of 0.5 or 1.0 gm. per kilo body weight, given in water solution, 20 cc. per kilo, with the addition of saponin in concentrations varying from 1.0 to 0.01%. Two preparations of saponin were used, Merck's purissum album and Morgan's. In control experiments carried out on the same animals one week previously, the calcium lactate solution was administered without saponin.

The method of determining the plasma calcium content was that of Kramer and Tisdall as modified by Tweedy and Koch[1] and by Pincussen and Schimmelpfeng.[2]

The initial blood calcium values in 48 experiments, showed a mean of 11.4 mg. ± 0.069 mg. The maximum reached in 24 control experiments was 13.65 mg. ± 0.157 mg. The maximum reached in 24 saponin experiments was only 13.92 mg. ± 0.134 mg.

There was no evidence that the duration of the rise in blood calcium was effected by saponin. If the blood calcium curve can be taken as an index of calcium absorption, the influence of saponin, in the dog, appears to be negligible.

Positive effects have been reported in studies on the isolated intestine of the guinea pig,[3] on mice,[4] and on man.[5] The recent studies of Wokes,[6] however, have failed to confirm some of the earlier work.

6238

A Method for Estimation of Both Bile Salts and Cholesterol in Small Amounts of Bile.

EDMUND ANDREWS AND LEO HRDINA.

From the Department of Surgery, University of Chicago.

In experimental and human studies on the chemistry of the bile, it often happens that only very small amounts of bile are available in the gall bladder, as the contents of the diseased viscus are frequently very scanty. It is in these very ones that analysis of the bile may be the most important. The following method enables one to determine both the bile acids and cholesterol in a single cubic centimeter of bile.

Following the suggestion of Dr. F. C. Koch the marked affinity of petroleum ether for bile salts was utilized. The principle is that petroleum ether has the power of making a quantitative separation of the bile acids and cholesterol in alcoholic solutions. The procedure is as follows:

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[1] Tweedy, W. R., and Koch, F. C., *J. Lab. and Clin. Med.*, 1929, **14**, 747.

[2] Pincussen and Schimmelpfeng, *Biochem. Z.*, 1927, **183**, 42.

[3] Lasch, F., *Biochem. Z.*, 1926, **169**, 301.

[4] Kofler, L., and Fischer, R., *Arch. f. exp. Path. u. Pharm.*, **130**, 319.

[5] Berger, F., Tropper, E., and Rischer, F., *Klin. Wochenschr.*, 1926, **5**.

[6] Wokes, F., *J. Pharm. and Exp. Ther.*, 1931, **43**, 531.

One cc. of bile is diluted with 6 cc. of alcohol, brought to a boil and filtered to deproteinize. The filtrate is then shaken in a separatory funnel with 30 cc. of petroleum ether and extracted 3 times. The alcoholic fraction is then warmed to drive out the petroleum ether and used for the bile acid determination. The ether fraction (about 100 cc.) containing the cholesterol is placed in an ordinary 37° incubator and will evaporate to dryness in 30 to 45 minutes, as its boiling point is 25° to 65°C. It is then dissolved in chloroform, made up to 5 cc. and used for an ordinary colorimetric cholesterol estimation. The pigments remain in the alcoholic fraction.

Thirty-five samples of bile, normal and abnormal from dogs and humans have been studied for controls of this technique. In no case could any test for bile acid be obtained in the ether fraction or for cholesterol in the alcohol fraction.

In 2 cases difficulty in separating the layers in the funnel occurred. This in each case was overcome by the addition of 2 drops of water. This added water of course stays in the alcohol fraction and must of course be taken into consideration in calculation of the bile acid content.

This method had been used in our laboratory before the paper of Elman and Taussig[1] was brought to our attention. They extracted the bile with hot alcoholic 3% KOH. Perhaps owing to the lighter quality of the petroleum ether used by us (B.P. 25-65), we have found the use of either heat or alkali unnecessary. It would, of course, partly hydrolyze the bile salts so that the combined analysis could not be made.

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[1] **Elman, R., and Taussig, J. B., *J. Lab. and Clin. Med.*, 1931, 17, 274.**

Minnesota Section.

University of Minnesota, May 25, 1932.

6239

Emptying of the Gall Bladder in Monkeys.

EDWARD A. BOYDEN.

From the Institute of Anatomy, University of Minnesota.

It has been clearly shown that in certain species of mammals, such as the opossum[1] and the rabbit,[2] there is little if any emptying of the gall bladder after meals, although Walsh reports that partial evacuation in the rabbit may be induced by cholecystokinin.[3]

It is of especial interest, therefore, to ascertain the rate of emptying of the gall bladder in animals nearest man. Through the courtesy of Dr. J. C. McKinley, 5 specimens of *Macacus rhesus* were made available for study, and the gall bladder of each visualized: first, by intravenous injection of tetraiodophenolphthalein, and afterwards, by direct introduction of lipiodol at the time of laparotomy.

Two of the animals (both of them females) failed to exhibit shadows after the Graham test and failed, subsequently, to empty the lipiodol after a meal of egg yolk. In one of these there was a supracolic peritonitis. In the other, as revealed by serial sections, there was a subacute or chronic cholecystitis with marked infiltration of plasma cells and lymphocytes, but with intact epithelium. This condition is of value as indicating what degree of inflammation is sufficient to prevent concentration of the dye.

In the remaining 3 animals (all males) the gall bladder was readily visualized and exhibited rapid evacuation of bile after a meal of egg yolk. The first one emptied half of its contents in the first 36 minutes (Graham method); the second one, ¾ of its contents in 24 minutes (Graham method), and all of its lipiodol in 32 minutes;

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[1] DuBois, F. S., and Hunt, E. A., *Anat. Rec.*, 1932, **52**, 11 (Supplement).

[2] Thompson, F. R., *Master's Thesis, Univ. of Minn.*, 1932.

[3] Walsh, E. L., *Proc. Am. Physiol. Soc.*, 1932, 101.

the third one evacuated most of its lipiodol in 27 minutes and disposed of the rest within the next 19 minutes. Nothing comparable to this initial rate of emptying has been found in any other species except man[4] and definitely establishes the supremacy of the primate gall bladder.

6240

Alterations of Protein Distribution Between Corpuscles and Plasma by Isotonic and Hypertonic Solutions.

C. J. BELLIS AND F. H. SCOTT.

From the Department of Physiology, University of Minnesota.

One of us (Scott[1]) observed that upon the addition of isotonic saline to whole blood *in vitro,* the total protein of the plasma was greater than in the original plasma; in other words, some protein had entered the plasma from the corpuscles. We have repeated these experiments and confirmed the observations as regards isotonic solutions, but when hypertonic solutions are used, the reverse occurs; there is a decrease in the total plasma protein, indicating that some protein had entered the corpuscles from the plasma. When hypertonic solutions were added to plasma alone, a decrease of protein did not occur, the total protein in the diluted specimen corresponding to the total in the control. Whether isotonic or hypertonic solutions were added to blood, the non-protein nitrogen of the plasma invariably increased.

The principle used was that employed by Scott. The corpuscle and plasma percentages were determined in duplicate by the hematocrit. The nitrogen determinations were made in duplicate by the modified micro-Kjeldahl method of Cavett,[2] including the use of H_2O_2[3] to complete the oxidation. 0.8 cc. of plasma was used for the total nitrogen determination and what would be equivalent to 2.5 cc. of plasma for the non-protein nitrogen, the protein-free filtrate being prepared according to the tungstic acid precipitation of Folin and Wu.[4] Duplicate blanks were run in all the determinations.

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[4] Boyden, E. A., *Anat. Rec.*, 1928, **40**, 147.

[1] Scott, F. H., *J. Physiol.*, 1915, **50**, 128.

[2] Cavett, J. W., *J. Lab. Clin. Med.*, 1931, **17**, 79.

[3] Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **56**, 2066.

[4] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **81**, 38.

Oxalated blood was used in all experiments except where the procedure involved the use of $CaCl_2$ as a diluent, in which case defibrinated blood was used. Similar results were obtained with either type of blood.

A typical experiment is outlined below:

Oxalated ox blood. 68.25% plasma.

0.8 cc. plasma = .0095598 gm. N of which .0002232 gm. is NPN.

∴ protein nitrogen = .0093366 gm.

90 cc. original blood was added to 10 cc. 50% dextrose solution and allowed to stand 21 hours (in most experiments 30 minutes).

Plasma = 74.735%.

0.8 cc. plasma = .00763 gm. N of which .0001984 gm. is NPN.

∴ protein nitrogen = .0074316 gm.

90 cc. original blood contained 61.425 cc. plasma, ∴ the total protein nitrogen = 61.425 × .0093366 × 10/8 = .7168911 gm.

Total non-protein nitrogen = .0171 gm.

In the diluted blood (plasma = 74.735%) the total protein nitrogen = 74.735 × .0074316 × 10/8 = .694213 gm.

Total non-protein nitrogen = .01853 gm.

∴ decrease protein N = .0226781 gm. = .1417 gm. protein.

∴ increase non-protein nitrogen = .0014 gm.

To make the results comparable, we have expressed them as if in each experiment we started with enough blood to have 100 cc. of plasma. The following table represents the protein and non-protein nitrogen changes as effected by the various solutions:

TABLE I.

Series	Diluent %	To Blood cc. cc.	Aver. Exp.	Protein change in Plasma gm.	NPN in mg.
1	50 dextrose	10:90	11	— .513	+ 3.9
2	” ”	25:75	1	— .143	+ 3.1
3	5.5 ”	10:90	2	+ .369	+ 1.2
4	” ”	20:80	2	+ .256	+ 1.1
5	” ”	50:50	2	+ .719	+ 3.9
6	” ”	70:30	2	+ .703	+10.4
7	8.1 NaCl	10:90	15	— .326	+ 2.5
8	0.9 ”	10:90	3	+ .243	+ 1.2
9	” ”	20:80	3	+ .576	+ 2.6
10	” ”	50:50	2	+1.603	+ 5.7
11	12.8 $CaCl_2$	10:90	1	— .029	+ 4.0
12	1.4 ”	10:90	1	+ .166	+ 1.2
13	” ”	20:80	1	+ .171	+ 2.0
14	” ”	50:50	1	+ .215	+10.2
15	” ”	70:30	1	+ .417	+18.9
16	2.65 $BaCl_2$ (isotonic)	10:90	2	+ .000	+ .1
17	” ”	20:80	2	+ .140	+ 1.2
18	” ”	50:50	2	+ .913	+ 5.1
19	” ”	70:30	2	+1.191	+11.0

+ = increase. — = decrease.

With the strength of hypertonic solutions used, it is not possible to dilute blood much more than 10% or some laking results. In the case of $BaCl_2$, 24% solution even in 10% dilution caused laking.

In the various experiments, the figures were all in the same direction. Thus, in the 11 experiments with hypertonic dextrose (Series 1), every experiment showed decreases of total protein which varied between .079 gm. and 1.913 gm. with an average decrease of .513 gm. In the 15 experiments using hypertonic NaCl (Series 7), the decreases varied between .052 gm. and 1.003 gm. with an average decrease of .326 gm. These phenomena are probably connected either with a reversal of charges on the corpuscles and plasma protein in concentrated solutions or with an alteration of the lipoid protein equilibrium between the cells and plasma. These possibilities are being investigated.

6241

Effect of Saliva on Coagulation of the Blood.

C. J. BELLIS, W. BIRNBAUM AND F. H. SCOTT.

From the Department of Physiology, University of Minnesota.

It was observed that the addition of human saliva to dog blood greatly shortened the coagulation time. Hunter[1] alone observed that the addition of his own saliva to his own blood hastened the coagulation. He did not try to determine the nature of the active substance. There is nothing specific about this material. Saliva hastened the coagulation of the following types of blood: (1) Other humans; (2) recalcified oxalated human blood of another individual; (3) dog blood; (4) recalcified oxalated ox blood; (5) recalcified oxalated ox plasma. Figures for dog blood are given below.

Human saliva was obtained from various individuals after rinsing the mouth with warm physiological saline and discarding the first samples. Salivary flow was increased by chewing paraffin. All samples of saliva were centrifuged for 30 minutes before using. Unless otherwise indicated, the saliva was unheated. When heated, the saliva was placed on a water bath for 20 to 45 minutes and then diluted to its original volume, cooled to room temperature, and filtered before using. Blood was obtained directly from the cannulated femoral artery of dogs into tubes containing the known amount of saliva. Four to 8 samples were used for each determination; the times given below are averages. The saliva and tubes were at room temperature. The times for coagulation are given in minutes and seconds.

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[1] Hunter, John B., *Brit. J. Surg.*, 1928, **16**, 203.

TABLE I.
Ratio of Saliva to Blood, cc. and Clotting Times.

Dog	Normal	0.6:2	0.4:2	0.1:2
1	6:00	1:00	2:35	4:30
2	5:30	1:30	2:00	3:30
3	2:00	:30	1:00	1:30
4	5:00	....	1:00	....
5	4:30	1:00	1:00	4:00
6	9:00	....	3:50	6:45

It will be seen that a reduction of 80% in clotting time can be obtained with proper proportions. Experiments in which saliva was added to blood in proportions of 1:400 showed a decreased coagulation time. When the proportion of saliva to blood is very great, such as 20:1, the coagulation time is still reduced, but not very much. Similar reductions in clotting time were observed in the case of the other types mentioned above. The clots formed after the addition of saliva seemed to retract more than normal blood.

Saliva may be heated to 60° with little reduction in coagulative power. Boiling, however, causes considerable reduction in power but it is not entirely destroyed by 5 minutes boiling over a free flame.

Saliva alone added to oxalate blood has little effect on the coagulation. If added in large amounts (equal or twice the volume of blood) some coagulation may be observed after a number of hours. We are inclined to believe this is due to the calcium in the saliva rather than to a thrombin, because if the saliva be oxalated before its addition to the blood no coagulation occurs. If we believe with Mills and Mathews[2] in the two types of coagulation, this would mean saliva contained no thrombin but a tissue fibrinogen or kephalin. However, as Loucks and Scott[3] showed, oxalates destroy thrombin. The nature of this phenomenon is being studied further.

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[2] Mills, C. A., and Mathews, A. P., *Arch. Int. de Physiol.*, 1924, **24**, 6.

[3] Loucks, M., and Scott, F. H., *Am. J. Physiol.*, 1929, **91**, 27.

6242

Effect of Ether and Iso-Amylethyl Barbiturate (Amytal) Anesthesia on the Glycogen Content of Skeletal Muscle.

S. G. MAJOR AND JESSE L. BOLLMAN.

From the Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minnesota.

The question of the glycogenolytic action of various anesthetic agents has received considerable attention in recent years, and although it is generally admitted that ether is a glycogen depleting agent, opinion on the influence of iso-amylethyl barbiturate (amytal) anesthesia remains divided. As a preliminary step in the investigation of the effect of various substances and procedures on the glycogen stores of the body, a series of experiments on the changes in the glycogen content of skeletal muscle under ether and amytal anesthesia was performed. Obviously, if specimens are to be removed under the influence of general anesthesia for the estimation of their glycogen content, the effect of the anesthetic agent on glycogen must be determined.

Brow and Long[1] showed that the glycogen content of heart muscle in fasted cats was reduced markedly with the closed mask method of ether administration, whereas there was only a slight reduction with the open method. Long,[2] employing the same species, showed that ether caused a distinct depression of the glycogen level of muscle. The results obtained by the majority of investigators concur with this work, although Hinsey and Davenport[3] have been unable to demonstrate any marked changes in the glycogen stores in skeletal muscle under the influence of ether anesthesia.

Amytal has become a popular anesthetic agent in recent years with investigators working on carbohydrate metabolism, because of the belief that it exercises a minimal effect on such metabolic processes. Page[4] showed that the blood sugar is not elevated beyond physiologic limits in dogs and rabbits under amytal anesthesia. Hines, Boyd and Leese[5] found that the substance did not alter the level of blood sugar in dogs, but that the ability to utilize glucose is impaired when this substance is injected by the continuous intravenous method. Hines, Leese and Barer[6] found the levels of muscle glycogen to be practically the same in dogs under the influence of amytal anesthesia and in control animals. Cori[7, 8] considered amytal to have so little influence on carbohydrate metabolism that it might be employed in tolerance experiments for injected glucose in rats. Although amytal in itself has such a slight effect, Cori thinks that it greatly intensifies the depressive action of epinephrine on utilization of glucose.

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[1] Brow, G. R., and Long, C. N. H., *Current Res. Anesth. and Anal.*, 1930, **9**, 193.

[2] Long, C. N. H., *J. Biol. Chem.*, 1928, **77**, 563.

[3] Hinsey, J. C., and Davenport, H. A., *Am. J. Physiol.*, 1929, **88**, 286.

[4] Page, I. H., *J. Lab. and Clin. Med.*, 1923, **9**, 194.

[5] Hines, H. M., Boyd, J. D., and Leese, C. E., *Am. J. Physiol.*, 1926, **76**, 293.

Opposed to the foregoing view are Best, Hoet and Marks,[9] Long, and Hinsey and Davenport, all of whom found that amytal lowers muscle glycogen in the cat.

Methods. The experiments here reported were all performed on dogs. The ether was administered by the intratracheal method, and the amytal by the intravenous route. Specimens of muscle which acted as controls were secured in a few cases from corresponding muscles of the opposite side of the body, and in other cases specimens were removed from the same muscle, care being taken not to injure the blood supply of the part in question. The glycogen determinations were done by Pflüger's method. The usual precautions in this procedure were taken.

Four experiments were performed to determine the effect of ether anesthesia on muscle glycogen. In all experiments there was a decrease in glycogen although the amount that disappeared was variable. A representative protocol is given.

Experiment 1. The dog weighed 23 kg. Ether anesthesia was started at 8:30 a. m. and was continued during the course of the experiment. The data are as follows:

Muscle	Time A. M.	Glycogen %
Right sartorius	9:13	0.942
" "	10:55	0.416
Left sartorius	11:13	0.384
Right gracilis	9:18	0.832
" "	11:18	0.207
Left gracilis	11:20	0.200
Right quadriceps	9:15	0.870
" "	11:15	0.437
Left quadriceps	11:16	0.439
Right adductor	9:20	0.870
" "	11:20	0.758
Left adductor	11:24	0.747

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[6] Hines, H. M., Leese, C. E., and Barer, A. P., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 736.

[7] Cori, C. F., PROC. SOC. EXP. BIOL. AND MED., 1925, **23**, 127.

[8] Cori, G. T., *Am. J. Physiol.*, 1930, **95**, 285.

[9] Best, C. H., Hoet, J. P., and Marks, H. P., *Proc. Roy. Soc. London*, 1926, **100**, 32.

Three experiments were performed to determine the effect of amytal anesthesia on muscle glycogen. A significant decrease in glycogen did not occur in any instance.

Experiment 2. The dog weighed 25 kg. During the procedure 58 mg. of amytal was administered intravenously. The glycogen values are as follows:

Muscle	Time A. M.	Glycogen %
Right sartorius	9:50	0.483
" "	11:55	0.551
Right gracilis	9:54	0.417
" "	12:01	0.591
Right quadriceps	9:52	0.344
" "	12:02	0.484
Right adductor	9:57	0.666
" "	12:05	0.680

We have shown repeatedly, as have numerous other investigators, that corresponding muscles of opposite sides of the body contain practically the same amount of glycogen. In the first protocol it is seen that the last 2 specimens of each group were taken almost simultaneously from opposite sides of the body, and in all cases there is fairly close correspondence in glycogen content. Furthermore, taking more than one specimen from a given muscle does not diminish its glycogen content provided there is no interference with the blood supply. In such a case the distal part of the muscle was used to obtain the first specimen, whereas the second specimen was removed from the proximal segment of the muscle.

Our results show that when ether is administered by the intratracheal method there is a coincident diminution of the glycogen content of skeletal muscle. On the other hand, muscle glycogen did not decrease significantly under anesthesia with amytal. On the basis of these experiments it would appear that amytal anesthesia is the anesthetic of choice in performing experiments involving muscle glycogen.

Summary. Corresponding muscles of opposite sides of the body contain similar amounts of glycogen. Prolonged ether anesthesia causes a marked decrease in the glycogen content of skeletal muscle. Amytal anesthesia causes little, if any, decrease in muscle glycogen content.

6243

Coagulative Power of "Activated" Chick Extract in Absence of the Calcium Ion.

JOSEPH T. KING. (Introduced by F. H. Scott.)

From the Department of Physiology, University of Minnesota.

It has been reported that the addition of 1 vol. rabbit plasma to 60 to 100 vol. chick extract greatly increases the coagulative power of the extract.[1]

According to the theory of Mills[2] there are two coagulants which may cause the clotting of blood fibrinogen, thrombin, which acts in the absence of ionized calcium, and tissue fibrinogen which causes coagulation only in the presence of the calcium ion.

The above criterion is utilized in an attempt to establish the nature of the substance causing the marked increase in the coagulative power of an extract which has been activated by the addition of a small amount of plasma.

Chick extract is made by extracting a 10-day chick embryo in 10 cc. of Ringer fluid. It is activated by adding 1 vol. citrated rabbit plasma to 20 vol. of extract. The sȧme extract without plasma addition serves as control. Coagulation time is determined as described in an accompanying paper.

The results with and without calcium are shown graphically in Fig. 1. The extract was activated at 11:35 and tested for coagulative power at subsequent intervals as indicated. The line labelled "Tissue Extract" shows the coagulative power of the control extract on recalcified citrate plasma; without recalcification of the plasma this extract causes no coagulation in 24 hours.

The line labelled "Tissue Extract Activated" shows the coagulative power of the activated extract on recalcified citrate plasma; the solid line gives the power of the same extract when the plasma is not recalcified.

Comparison of the power of the activated and control extract, in the presence of the calcium ion, shows that the former is much more powerful than the latter. According to the work of Mills[3] a decrease in coagulation time from 1¼ minutes to 20 seconds indicates an increase in the concentration of the coagulant of about 64 times.

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[1] King, J. T., *Arch. f. Exp. Zellforsch.*, 1931, **10**, 467.

[2] Mills, C. A., *Chinese J. Physiol.*, 1927, **1**, 435.

[3] Mills, C. A., *Am. J. Physiol.*, 1922, **60**, 193.

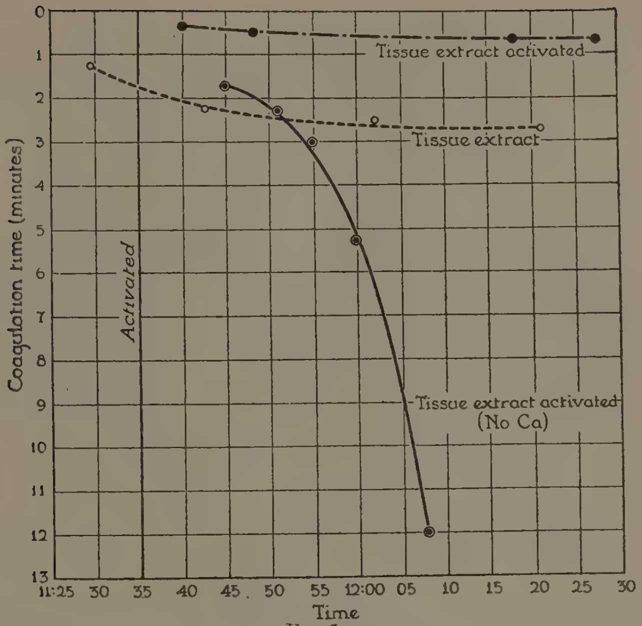

FIG. 1.

The coagulative power of "activated" chick extract in the absence of the calcium ion.

The fact that the activated extract shows coagulative power on citrate plasma without recalcification, whereas the control extract shows no clotting in 24 hours, indicates that a coagulant has appeared in the process of activation which, like thrombin, can cause coagulation independent of the presence of the calcium ion.

The rapid decrease in the coagulative power of the activated extract in the absence of calcium is consistent with the work of Loucks and Scott[4] showing that thrombin is rapidly destroyed in the presence of oxalate. I have found (unpublished experiments) that citrate acts similarly. It must be assumed that the coagulant is exposed to a citrate excess in these experiments (when the plasma is not recalcified) since normal extract fails to show coagulation in 24 hours under these conditions.

The above data indicate that the coagulant involved in the process of activation behaves like thrombin in relation to the calcium ion.

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[4] Loucks, M. M., and Scott, F. H., *Am. J. Physiol.*, 1929, **91**, 27.

6244

A Method for Distinguishing Between "Activation" and Addition Effects when Mixing Coagulants.

JOSEPH T. KING. (Introduced by F. H. Scott.)

From the Department of Physiology, University of Minnesota.

Mills[1] reported results obtained on mixing serum and lung extract; in general there was noted an increase followed by a decrease in the coagulative power of the mixture. He particularly emphasized the tremendous increase in coagulative power that occurs immediately after mixing. He used the decrease in the clotting time of a recalcified citrate plasma as an index of coagulative power.

Burns, Scharles and Aitken[2] reported a study on a series of kidney extracts from different animals incubated with various sera. Using the decrease in clotting time of heparinized plasma as an index of coagulative power, they secured results somewhat similar to those obtained by Mills with lung extract.

Considerable interest attaches to the interpretation of this phenomenon in terms of coagulation theory. In order accurately to determine whether activation occurs and the extent of the activation where it does occur, it is necessary to use a method which will measure activation at its height (according to available data this is immediately after mixing). And since serum as well as tissue extract has coagulative power, the method used should permit distinguishing between addition and "activation" effects.

The following method is found to meet the above requirements. Decrease in the clotting time of recalcified citrate rabbit plasma is used as the index of coagulative power. Coagulation time is determined in 10 mm. tubes kept in a water bath at 30°C. The materials are added in the following order. Plasma, 2 drops; 1% $CaCl_2$, optimum amount; coagulant; 0.9% NaCl to make 10 drops. The strength of the coagulants is now adjusted, by dilution of the stronger with saline, until 2 drops of either decrease the clotting time of the recalcified citrate plasma to the same extent. The mixed coagulants are now tested by adding to the tube, plasma, 2 drops; $CaCl_2$, optimum amount; 1 drop of each coagulant, NaCl to 10 drops. Activation will be indicated by shortening of the coagulation time beyond that caused by 2 drops of either coagulant alone.

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[1] Mills, C. A., *Am. J. Physiol.*, 1922, **60**, 193.

[2] Burns, E. L., Scharles, F. H., and Aitken, L. F., *Am. J. Physiol.*, 1931, **97**, 233.

In a typical experiment, serum mixed with tissue fibrinogen (Merrel's Fibrogen) shows no (or occasionally just a trace of) activation. The following values were obtained: 2 drops serum, coagulation time 3¼ min.; 2 drops tissue fibrinogen, 3¼ min.; 1 drop of each, 3¼ min. The same serum, used in greater concentration to render it isocoagulant with fresh chick extract, shows marked activation; 2 drops serum, 2¼ min.; 2 drops chick extract, 2¼ min.; 1 drop of each, 45 sec.

Controls are run before and after the experiment to determine whether the coagulants remained isocoagulant during the test. Plasma is citrated to 0.5%; serum is from citrate plasma freshly clotted by recalcification; chick extract is made by extracting a 10 day chick embryo in 10 cc. Ringer fluid according to a method described earlier.[3]

6245

Serum Calcium in Relation to Menstruation in Cases with Dysmenorrhea.*

RUTH E. BOYNTON AND ESTHER M. GREISHEIMER.

From the Students' Health Service and Department of Preventive Medicine and Public Health and the Department of Physiology, University of Minnesota.

A study of the serum calcium in relation to the menstrual cycle in a group of 10 women who had dysmenorrhea was made. The cases selected were those having severe dysmenorrhea necessitating bed rest every month. Our purpose was to determine whether women having dysmenorrhea showed any significant variations in calcium level from women with normal menstrual periods.

Serum calcium determinations were made in duplicate on 10 dysmenorrhea cases twice a week for 4 consecutive weeks. The blood was drawn between 8 and 10 a. m. The determinations of serum calcium were made by one of us (E. M. G.) using the Clark-Collip[1] modification of the Kramer-Tisdall[2] method. A total of 80 readings were made.

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[3] King, J. T., *Arch. f. Exp. Zellforsch.*, 1930, **9**, 341.

* This study was carried out with the aid of a grant from the Research Fund of the University of Minnesota.

[1] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

[2] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **47**, 475.

TABLE I.
Serum Calcium Variations in a Group of 10 Cases of Dysmenorrhea. Bi-weekly Determinations for 4 Weeks.

Case No.	Age	Low	High	Mean	P. E. of Mean	S. D.
1	18	10.50	11.35	10.81	±.07	.29
2	21	9.82	10.75	10.34	±.08	.33
3	23	10.29	10.93	10.54	±.05	.20
4	22	10.52	10.73	10.64	±.02	.07
5	21	10.33	10.62	10.49	±.02	.10
6	22	10.38	11.08	10.73	±.06	.27
7	19	10.56	10.97	10.78	±.04	.14
8	20	10.56	11.05	10.76	±.05	.20
9	22	9.59	11.06	10.09	±.11	.45
10	30	9.73	11.52	10.53	±.13	.51
Means	21.80	10.23	11.01	10.57		
			Range .78			

Table I presents the range in the serum calcium and the mean of the 8 determinations for each individual in the group.

The mean low reading for the group was 10.23 mg., the mean high reading 11.01 mg., giving a mean range of 0.78 mg. In a group of women with normal menstrual periods studied by us,[3] the range was 1.15 mg. This difference in range, however, is not sufficient to be of significance.

The mean reading for the entire group was 10.57 mg. In our group of normal women we found a mean of 10.01 mg., again a difference of no significance.

TABLE II.
Serum Calcium Level in Relation to Menstrual Cycle, 10 Cases of Dysmenorrhea.

	Mean mg. Calcium	P. E. of Mean	S. D.	Difference of Means
Rest	10.49	±.05	.3042	
				.08±.06
Pre-menstrual	10.57	±.04	.2769	
				.04±.07
Menstrual	10.61	±.05	.3319	
				.00±.08
Post-menstrual	10.61	±.06	.4277	
				.12±.08

Table II presents the mean serum calcium for each week of the menstrual cycle. The lowest reading of 10.49 mg. occurs in the rest period, while in the normal group, the lowest reading occurred in the menstrual week. The apparent cyclic variation in calcium levels in relation to menstruation found in the women with normal menses, with a pre-menstrual rise and post-menstrual fall in serum calcium was not observed in this group with dysmenorrhea. No significant differences between the mean reading for each week of the menstrual cycle was found.

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[3] Boynton, Ruth E., and Greisheimer, Esther M., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 907.

All of the cases of dysmenorrhea in this group had previously been treated by the oral administration of calcium gluconate. Cases 1 to 6 inclusive had complete relief with calcium therapy while cases 7 to 10 had no relief. In Table III the mean calcium readings in relation to the menstrual cycle for the group of cases relieved by calcium therapy and for the group without benefit are presented. No significant differences are found in the 2 groups at any period in the cycle. It is evident that no basis for predicting response to calcium therapy can be obtained by serum calcium determinations.

TABLE III.
Serum Calcium Level in Relation to Menstrual Cycle.
A—6 Dysmenorrhea Cases Relieved by Calcium Therapy.
B—4 Dysmenorrhea Cases not Relieved by Calcium Therapy.

	A			B		
	Mean	P. E.	S. D.	Mean	P. E.	S. D.
Rest	10.56	±.05	.27	10.38	±.11	.45
Pre-menstrual	10.60	±.05	.27	10.52	±.08	.29
Menstrual	10.58	±.03	.12	10.64	±.11	.48
Post-menstrual	10.62	±.07	.36	10.59	±.12	.50

Summary. From a study of the serum calcium of 10 cases of dysmenorrhea biweekly for 4 weeks, it is found: 1. The mean range of serum calcium, while lower than that found in a group of women with normal menstrual periods, was not significantly lower. 2. The lowest mean serum calcium occurred in the rest period of the menstrual cycle. 3. In women with dysmenorrhea there appears to be no cyclic variation in calcium level in relation to the menses. 4. There seems to be no significant difference in serum calcium levels in cases of dysmenorrhea responding to calcium therapy and in those not benefited by such therapy.

6246

Effect of Operation on Persistence of Inspired Bacteria in the Lungs of Mice.

M. VON JACOBI* AND HERBERT A. CARLSON.
(Introduced by O. H. Wangensteen.)

From the Department of Surgery, University of Minnesota.

Both the bronchogenous and embolic theories of the pathogenesis of postoperative pneumonia command attention at the present time. The rôle played by bacteria already present in the respiratory tract or introduced into the body at the time of operation must be evaluated. It is probable that bacteria may enter the lungs through the air passages, that they may be removed in the lungs by some defense mechanism, and that certain factors may inhibit this defense of the lungs against the inhaled bacteria. Such a conclusion seems to be justified by the work of Stillman,[1] who subjected mice to atmosphere laden with various bacteria; when pneumococci Type I were inspired by normal mice they could later be demonstrated in the lungs, but disappeared within 3 hours. However, when alcoholized mice were subjected to the same experiment pneumococci were found in the lungs as late as 5 days after breathing the contaminated air.

Because of these facts we were led to investigate the disappearance or persistence of inspired microorganisms in the lungs of mice after operation in order to determine whether there was any delay in the removal of bacteria which might be a factor in the pathogenesis of pneumonia.

Method. The general technique was similar to that described by Stillman.[2] Mice were placed in a metal box into which broth cultures of bacteria were sprayed. Some of these mice were normal controls. Others had had operations which consisted of simple laparotomy and closure or simple incision and closure of the skin and muscles of the thorax. These procedures were carried out under novocaine infiltration anesthesia. After having been sprayed for one hour the animals were removed from the spraying chamber. They were sacrificed at definite intervals of time by strangulation and then were immersed in lysol solution for 10 minutes prior to necropsy. The thorax was opened with an electrocautery and sections of the hilus and periphery of the lung removed by means of sterile instruments for culture. A pneumococcus Type I culture was employed in the spray in the early experiments, but because of difficulty in identifying this organism and because of the inhibiting effect on it of other bacteria, the experiments were repeated with *B. prodigiosus* and Friedländer bacillus. In order to identify the pneumococci we routinely used blood agar plate and veal broth, thereby obtaining simultaneously the advantages of a separating medium as well as one upon which the pneumococcus grew readily. As a final test the bile solubility test was employed.

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* Exchange assistant from Prof. de Quervain's Clinic in Berne, Switzerland.

[1] Stillman, E. G., *J. Exp. Med.*, 1923, **38**, 117.

[2] Stillman, E. G., and Branch, A., *J. Exp. Med.*, 1924, **40**, 733.

Results. The results are recorded in the following tables, the time interval referring to the time after removal from the spraying chamber, and the terms positive and negative indicating whether the sprayed organisms could be cultured from the lungs. (Tables I, II, and III.)

TABLE I.
Sprayed with Pneumococcus Type I.

A Controls	Hours	1	2	3	4	5	6	7	Total mice
	Positive	2	1	2	3	2	0	—	
	Negative	4	5	4	3	4	10	—	40
B Abdominal Operations	Positive	—	—	—	0	0	0	0	
	Negative	—	—	—	2	2	5	9	18
C Thoracic Operations	Positive	—	—	—	0	0	0	0	
	Negative	—	—	—	1	1	1	6	9

TABLE II.
Sprayed with *B. prodigiosus.*

A Controls	Hours	1	2	3	4	5	6	7	Total mice
	Positive	3	0	2	0	1	0	—	
	Negative	0	3	1	3	2	5	—	20
B Abdominal Operations	Positive	—	—	—	2	1	1	0	
	Negative	—	—	—	0	1	4	9	18
C Thoracic Operations	Positive	—	—	—	0	1	0	0	
	Negative	—	—	—	1	0	1	6	9

TABLE III.
Sprayed with Friedländer Bacillus.

A Controls	Hours	1	2	3	4	5	6	7	Total mice
	Positive	3	2	0	1	0	0	—	
	Negative	0	1	3	2	3	5	—	20
B Abdominal Operations	Positive	—	—	—	0	2	0	0	
	Negative	—	—	—	2	0	5	9	18
C Thoracic Operations	Positive	—	—	—	1	0	0	0	
	Negative	—	—	—	0	1	1	6	9

The inspired organisms were recovered irregularly during the first 5 hours. Only one culture of a mouse operated upon was positive at the end of 6 hours (Table II, B). Although more positive cultures were obtained in the animals operated upon than in the controls 4 or 5 hours after the spraying with *Bacillus prodigiosus* or with Friedländer bacillus, this result was not striking and was contradicted by the experiments with pneumococcus.

Contaminating organisms were present in 29 of 40 cases, particularly the subtilis, micrococcus and pyocyaneous groups which in many instances undoubtedly inhibited the growth of the organisms being studied, and prevented their demonstration (Neisser[3] and Jones[4]). This we were also able to demonstrate by mixing cultures of contaminating bacteria with pneumococci and attempting to reisolate the pneumococci. The presence of contaminating bacteria is undoubtedly one of the factors contributing to the irregularity of the recorded results.

Summary. 1. The examination of 80 mice showed that the inhaled pneumococci, *B. prodigiosus* and Friedländer bacilli can remain 5 hours in the lungs. 2. The microorganisms are found in the lung irregularly from one to 5 hours after inhalation. 3. There was no difference between the peripheral and hilus portion of the lungs. 4. Examination of 81 mice showed that abdominal or thoracic operations produced no increase in the period of persistence of the inhaled bacteria in the lungs. 5. No definite evidence was found to justify the belief that surgical operations handicap the mechanism by which bacteria are destroyed or removed from the lungs of mice.

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[3] Neisser, M., *Z. f. Hyg. u. Inf. Krankheiten.*, 1896, **5**, 12.

[4] Jones, F. S., *J. Exp. Med.*, 1922, **36**, 317.

Missouri Section.

St. Louis University School of Medicine, May 11, 1932.

6247

Origin and Structure of a Fibrin-Type in Normal Blood.

JOHN AUER.

From the Department of Pharmacology, St. Louis University School of Medicine.

If normal blood from the rabbit and guinea pig is washed and centrifuged 4 to 6 times in 20 times the blood volume of Ringer or Ringer-saline solution, the same type of fibrin-filaments attached to red corpuscles may be observed as were previously described.[1] These filaments appear within 25 minutes to 2 hours after the hanging-drop preparation has been made, and exhibit the same functional manifestations that were observed in ordinary blood hanging-drops. These filaments therefore have their origin in the red corpuscles themselves, and probably arise from the fibrinogen-like substance which Horino[2] isolated from washed red corpuscles.

The ordinary fibrin threads of plasmatic origin forming about platelets do not exhibit the phenomena described for the fibrin filaments attached to red corpuscles. Moreover the addition of sodium oxalate, hirudin or heparin in sufficient amounts to largely abolish the formation of plasmatic fibrin, had only little effect on the formation and activity of the fibrin-filaments from red corpuscles.

Observation of the fibrin-filaments issuing from red corpuscles shows in favorable instances that it is composed of fibrils which are twisted about each other like the strands of a rope. Rupture of such a fibril brings about the formation of small pellet which may rotate around the main stem with gradually increasing radius until it swings free on its own pedicle from the base of the main filament. Such pellets may again twist themselves around the parent stem in a spiral manner and disappear. The largest number of such pellets observed on one ruptured filament was 5. These fibrils are in turn composed of invisible (bright-light field) sub-fibrils. The presence of such invisible sub-fibrils is demonstrated by their distorting effects on red corpuscles, by the alternate disappearance and reappearance of sections of the filament, and by the sudden movement of apparently free corpuscles or microcytes.

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[1] Auer, J., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 618.

[2] Horino, K., *J. Biochem.*, 1928, **9**, 437.

The rotation or twisting of the fibrin filament is beautifully demonstrated at times by the rotation of a corpuscle stretched out between 2 filaments; the corpuscle changes from a view on the flat to a view on edge and back again.

6248

Loss of Blood as a Factor in Death from Acute Portal Obstruction.

ROBERT ELMAN AND WARREN H. COLE. (Introduced by Evarts A. Graham.)

From the Department of Surgery, Washington University Medical School, and Barnes Hospital, St. Louis, Missouri.

Sudden occlusion of the portal vein is followed very rapidly by shock and death within a few hours. The reason has never been explained. The blood pressure falls within a few minutes and soon drops to a shock level and continues downward until exitus. A decrease in the thrombocyte count and in clotting time has been recorded recently.[1] With the idea that a toxemia similar to that assumed to occur in low intestinal obstruction and strangulation might be present, we examined the lymph from the thoracic duct, the systemic blood and the portal blood from animals before and after portal ligation. By injecting the material into guinea pigs no differences in toxicity were observed.

Sufficient blood may have been lost from the general circulation into the engorged mesenteric veins and intestinal capillaries in dogs with obstruction of the portal vein to account for the rapid death. To investigate this both cats and dogs were used. Pairs of the same age, weight and sex were selected as far as possible and starved for 24 hours. The same anesthetic was employed and similar laparotomy at the same time was performed in each pair of animals. A ligature was placed around the lower rectum in each to prevent escape of intestinal contents. The portal vein was isolated in each and was tied in one. As soon as the one with ligation died the control was killed. The entire gastro-intestinal tract of each from the

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[1] Tschernikoff, A. M., Malenjuk, W. W., Jakubovitsch, M. I., and Klantaroff, M. Ch., *Arch. f. d. ges Physiol.*, 1931, **227**, 85.

TABLE I.

	Controls			Portal Ligation			
	Wt. Animal (kg.)	Wt. Guts (gm.)	% B.W.	Wt. Animal (kg.)	Wt. Guts (gm.)	% B.W.	% Increase over control
Average 4 dogs	8.07	611	7.6	6.60	854	12.8	5.2
Average 6 cats	2.65	165	6.3	2.94	282	9.7	3.4

cardia to the anus was removed *in toto* including the spleen and with avoidance of the loss of fluid from them. The results are shown in Table I and are expressed in terms of weight of intestinal tract as percentage of the body weight. By subtracting the weight of the intestinal tract of the controls from that of animals with portal obstruction one obtains a figure which represents the amount of fluid and blood lost into the gut. In the case of the dogs this represented an average of 5.2% of the body weight. This is above the amount necessary to kill dogs by simple hemorrhage as shown by many observers, and particularly by Johnson and Blalock.[2] In cats the amount lost in the intestinal tract was an average of 3.4% of the body weight which is also sufficient to cause death from simple hemorrhage. We have observed death following the loss of 2.7% of the body weight of a few control cats by simple bleeding from the femoral artery.

The behavior of animals after portal ligation is similar to that caused by severe loss of blood from hemorrhage. They become increasingly weak, respirations become rapid and shallow with occasional deep inspirations indicating "air hunger", and thirst seems apparent from their repeated attempts to find water in the empty crocks in the cage. Though prostration becomes increasingly severe, the sensorium is unaffected up to the end. There are no convulsions. At autopsy the tissues of the body are obviously dry and quite bloodless. Section of the intestine reveals tremendous engorgement of the capillaries. Sometimes but not always there is a transudation of bloody fluid into the lumen of the bowel. There is very little peritoneal fluid. Scott and Wangensteen[3] recently reported considerable fluid and blood loss from the general circulation after strangulation or ligation of the veins of a length of intestine. They believe this loss was sufficient to cause death.

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[2] Johnson, G. S., and Blalock, A., *Arch. Surg.*, 1931, **22**, 626.

[3] Scott, H. G., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 748.

6249

Transmission to Offspring of Immunity Against Infection with a Metazoan (Cestode) Parasite.*

HARRY M. MILLER, JR.

From the Department of Zoology, Washington University.

In view of the successful results of numerous experiments on passive transfer of immunity[1] against infection with onchospheres of *Taenia taeniaeformis,* it was not surprising to find that a degree of protection was transmitted to the offspring of female rats actively immunized against this worm or infected with the larval stage. More than 250 such young rats have been used in a series of experiments begun in 1930.

The young of immune and control (stock) rats were infected at weaning, or at varying intervals thereafter, by feeding, by means of a stomach tube, equal portions of a uniform suspension of infective onchospheres. In most such instances cysts (2 to 5 mm. diameter) were present in the liver of control animals at autopsy 4 to 5 weeks later, while cyst development was completely, or almost completely, inhibited in the offspring of infected or immunized females. The results of a typical experiment are shown in Table I.

TABLE I.
Average number of living and dead cysts in liver at autopsy.

Infected Mothers No.	Cysts in Liver of Mother	Birth Date	Offspring† Number	Living Cysts	Dead Cysts
24470	30	1/16	6	3	6
23677	31	1/16	5	—	—
16(78)	220	1/14	9	—	—
Controls	—	1/16	8	35	0.5

† Infected February 12, autopsied March 12, 1932.

In these experiments protection of the young of infected females was greater than that of the offspring of actively immunized females. In either case this immunity is passive and has not been found to persist beyond about 6 weeks after birth.

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* This investigation was in large part made possible by a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Miller and Gardiner, *Science,* 1932, **75**, 270; PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 779.

6250

Acquired Immunity Against a Metazoan Parasite by Use of Non-Specific Worm Materials.*

HARRY M. MILLER, JR.

From the Department of Zoology, Washington University.

Both active and passive immunization of the rat against infection by onchospheres of *Taenia taeniaeformis* have been adequately demonstrated.[1] A series of 6 injections of a 1% suspension of the powdered worm usually results in complete protection against infection. Only one attempt, previous to the present experiments, was made to produce immunity by the use of related worm materials; injection of powdered *T. pisiformis,* a tapeworm of the dog, did not protect rats against infection by larvae of *T. taeniaeformis.*

In experiment I powdered *Diphyllobothrium latum, Taenia saginata, Hymenolepis sp.,* and *Dipylidium sp.* were used. One lot of control rats received a similar series of injections of *T. taeniaeformis* material and another (uninjected) served as controls on the viability of the *taeniaeformis* onchospheres with which all rats were fed. Autopsy data (Table I) show that little or no protection was

TABLE I.
Figures give the average number of cysts in liver.

Exp. No.	Material used	Method	No. of Rats	Living Cysts	Dead Cysts
1	*Diphyllobothrium latum*	Intraperitoneal	8	173	8
	Taenia saginata	"	9	104	38
	Hymenolepis sp.	"	10	145	20
	Dipylidium sp.	"	9	168	23
	Taenia taeniaeformis (controls)	"	10	21†	17
	Untreated controls	—	10	206	7
2	Living *Cysticercus fasciolaris*	Placed in body cavity	7	—	2
	Living *Taenia pisiformis*	"	6	15	22
	Untreated controls	—	7	214	12
3	Onchospheres of *T. pisiformis*	Fed once	9	152	19
	"	Fed twice	9	100	31
	Untreated controls	—	12	215	8

† No living cysts in 7 rats.

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* This investigation was made possible by a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Miller, H. M., Jr., PROC. SOC. EXP. BIOL. AND MED., 1930-1932, **27**, **28**, **29**; *J. Prev. Med.,* 1931, 1932, **5**, **6**; and papers in press.

afforded by these non-specific cestode materials, following their injection as suspensions of powdered worm.

On the other hand, introduction into the peritoneal cavity of rats of whole, or long pieces of, living *Taenia pisiformis* resulted in a high degree of protection against infection by the larvae of *T. taeniaeformis,* as was shown by infecting the rats some weeks after the operations (Exp. 2). The control animals into whose body cavities the cysticercus stage of *taeniaeformis* had been placed contained no living cysts at autopsy, while the untreated controls were heavily infected.

On one earlier occasion the mistake had been made, in one experiment, of feeding onchospheres of *T. pisiformis* instead of *T. taeniaeformis.* When these rats were subsequently fed onchospheres of the proper species it was found that the control (untreated) animals were refractory to infection, although control rats of other experiments to which portions of the same lot of onchospheres had been fed became heavily infected. To test the hypothesis that feeding with eggs of one species would confer protection against infection with those of another, in this case, closely related, species, rats of one group (Exp. 3) were fed once with 1500 *pisiformis* onchospheres, and those of a second group were fed again, 3 weeks later, with approximately 9000 onchospheres each. These anmials, together with brother and sister (untreated) controls, were fed *taeniaeformis* onchospheres several weeks later. The autopsy data are shown in the table.

6251

Studies on Blood Diastase.

MICHAEL SOMOGYI.

From the Laboratory of the Jewish Hospital of St. Louis.

Diastase is known to be a normal constituent of human blood, but available information as to its quantity is inconsistent. The variety of measuring units and the multiplicity of methods render it impossible to correlate results and to explain conflicting conclusions. Even one and the same technique yields different results in the hands of different workers. One factor responsible for this situation is the use of soluble starch as substrate in measuring the enzyme action. It is practically impossible to prepare 2 identical batches of soluble starch, and consequently each will give different results with the same quantities of the enzyme, the discrepancies frequently being quite considerable. Moreover, investigators in general fail to consider the intricacies of the kinetics of diastase action and carry out their determinations under conditions where the amount of reaction products determined is not in linear proportion to the amount of enzyme.

A study of substrates convinced us that starch pastes prepared from various refined natural starches by boiling at atmospheric pressure furnish adequate substrates. Pastes of 0.5 to 2% concentration, prepared from well washed rice, corn, wheat, potato or arrowroot starches, yield identical amounts of reducing sugars if incubated with identical amounts of enzyme under standardized conditions. It is remarkable that with glycogen the same results are obtained as with starch pastes. In another approach to the quantitative determination of diastase, in the measurement of the rate of cleavage of starch to the point where it no longer gives blue color with iodine, all starch pastes studied yield reproducible results, while every batch of soluble starch differs from the other. Thus the use of starch paste as substrate eliminates one variable in the study of diastatic activity. The next step was to determine and standardize the conditions which insure a direct proportionality between the amount of enzyme and that of reaction products. This is feasible by proper adjustment of the relative concentrations of enzyme and substrate.

The 2 phases of enzyme action, the amyloclastic and the saccharifying, exhibit a fair parallelism so that both are equally useful as the basis of quantitative methods. The technique involving the determination of the sugars, however, is more accurate, as the judgment of color changes in the starch-iodine reaction is more or less subjective. To diminish the influence of the subjective factor, we have applied the observation of the transparency of the reaction mixture as an additional criterion in establishing the rather arbitrary end point.

In the examination of numerous blood samples obtained from healthy humans, we find that the bulk of the diastase is in the plasma, the corpuscles containing less than one-fifth of the total. The normal diastase content of human blood plasma exhibits variations of 100%, while in toxemic conditions it is in general greatly diminished.

Cleavage products of starch under the effect of blood diastase were also studied. Contrary to statements in the literature, we find but very small quantities of glucose and maltose. The bulk of the reducing sugars produced by blood diastase is a non-fermentable substance which we were able to isolate in crystalline form. It possesses approximately one-third of the reducing power of glucose, and preliminary examination indicates that it is a trisaccharide (Lohmann, Barbour). By alcoholic fractionation another crystalline polysaccharide was separated which does not reduce copper, gives no color reaction with iodine and is probably identical with Pringsheim's "graenzdextrine," a tetra- or hexa-hexosan. Further analysis of the 2 substances is in progress. The generally assumed presence of the enzyme maltase in blood found no substantiation in our experiments.

6252

Effects of Anterior Pituitary from Various Species on Sex and Thyroid of Immature Guinea Pigs.*

LEO LOEB.

From the Department of Pathology, Washington University School of Medicine.

We have shown that different preparations of the anterior pituitary gland of different species, as well as urine of pregnant women, exert on the sex organs of immature guinea pigs varying effects. The differences were not equally marked in all cases; they were quite definite in the following 3 groups: (a) various preparations of anterior pituitary of cattle; (b) urine of pregnant women, and (c) anterior pituitary of guinea pig, rabbit and cat. Within the latter group (c) the differences between cat and rabbit were of a quantitative character, rabbit anterior pituitary showing somewhat greater effects than cat anterior pituitary; otherwise they were essentially the same. The effects of anterior pituitary of guinea pig differed from those of anterior pituitary of rabbit and cat, in that in the former the development of large mature follicles was very prominent, while the production of pseudolutein bodies and interstitial gland was relatively insignificant in contrast to the latter, in which the pseudoluteinizing effects were much more marked. Our attempts to make the effects of the anterior pituitaries of rabbit and guinea pig identical by varying the quantities of both, were not quite successful.

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* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

We concluded therefore that, while quantitative differences in the concentration of the active principles in the anterior pituitary of rabbit and guinea pig were partly responsible for the results obtained, there might be in addition a difference in the relative strength of the principle supposed to promote the growth and maturation of follicles on the one hand and of the pseudoluteinizing agent on the other hand. To investigate further, we carried out a new series of experiments in which we varied to a still greater extent the quantities of anterior pituitary glands of different species inoculated into immature guinea pigs; we also tested the effects of rat anterior pituitaries. The number of guinea pig anterior pituitaries inoculated varied in different experiments between one and 6. The quantities of rabbit anterior pituitaries used varied between one and one-third of a gland. In former experiments we had used in addition 2 anterior pituitaries of rabbits. The number of rat anterior pituitaries inoculated varied between 1 and 4. Altogether it was necessary to use in this second series the anterior pituitary glands of 148 rats, 182 guinea pigs and 76 rabbits. They were inoculated into 44 immature guinea pigs. These figures indicate that the number of experiments of this kind which can be carried out is limited. We also extended our investigations concerning the effects of the other 2 groups of substances, anterior pituitary of cattle and urine of pregnant women, by further varying the quantities.

Our further experiments confirmed our conclusions concerning the differences between the main 3 groups, (a) anterior pituitary of cattle, (b) of rabbit and guinea pig, (c) urine. We have not found it possible to make the effects between these groups equal by varying the quantities used. Our experiments indicate that the differences between the effects of rabbit, guinea pig and also rat anterior pituitary glands depend essentially on the concentration of the active principle or principles present in these organs. However, these concentrations differ considerably in the different species. Inoculation of one-third to one-half of a rabbit anterior pituitary (weighing approximately between 4-6 mg.) is about equal in effect to inoculation of 6 anterior pituitary glands of the guinea pig weighing about 44.5 mg. The inoculation of 6 anterior pituitaries of guinea pig is approximately equal in its effects to inoculation of 1 rat anterior pituitary weighing approximately 3 mg.†

This similarity in the action of the pituitaries of these 3 species relates, first, to the changes which take place in the ovary. Here with increase in the quantity of anterior pituitary the pseudolutein bodies and interstitial gland become relatively more prominent, although mature follicles may still continue to be produced. However, with an increase in the number of rat anterior pituitaries, daily inoculated, to 4, the number of mature follicles decreases, perhaps owing to the fact that the increase in hypertrophy and activity of the thyroid gland, which was parallel to the increase in rat anterior pituitaries used and its unfavorable influence on the weight of the animals, exerted a depressing effect on the maturation of follicles.

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† We are indebted to Miss Hilda Friedman for determining the weights of the various kinds of anterior pituitary glands.

Second, the similarity relates also to the condition of the vagina. After inoculation of one anterior pituitary of rabbit or rat, the vaginal epithelium did not usually undergo full proliferation, although there were fully mature follicles present. On the contrary after daily inoculation of one to 5 guinea pig anterior pituitaries typical squamous epithelium developed in the vagina; but after daily inoculation of 6 anterior pituitaries of the guinea pig, the vagina underwent only a very rudimentary proliferation. In accordance with our previous conclusions[1] we may assume that the presence of pseudolutein bodies and of interstitial gland or of either of these 2 kinds of structures inhibits the full proliferation of the vagina. On the other hand, in case the maturation of follicles was prevented by underfeeding, vagina, uterus and mammary gland were usually found in a resting condition.

In our former series ovulation occurred mainly after inoculation of guinea pig anterior pituitaries and only exceptionally after inoculation of the anterior pituitary of other species. In this series we found also some ovulations taking place after inoculation of rabbit as well as of rat anterior pituitaries. But in the latter cases the ovulation occurred only in guinea pigs weighing over 200 gm., while in the former case it occurred in young animals in the majority of cases. Further investigations must show whether this difference is of significance. Furthermore we obtained ovulation only after daily inoculation of 3 mg. of rat anterior pituitary and not after inoculation of 12 mg. of this material, corresponding to 4 rat anterior pituitaries, while we obtained ovulation after daily inoculation of one as well as of 4 guinea pig anterior pituitaries varying in quantity approximately between 7 and 30 mg. It is probable that the presence of large amounts of pseudolutein tissue and of interstitial gland is an unfavorable factor as far as the occurrence of ovulation is concerned. If after inoculation of anterior pituitary glands ovulation does take place, the typical changes in the ovaries consisting in an almost complete degeneration of all except the smallest follicles and in the marked proliferation of the vaginal epithelium were in a number of cases more or less inhibited.

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[1] Loeb, Leo, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 642; *Endocrinol.*, 1932, **16**, 129.

The anterior pituitary of the guinea pig is very inactive also in its effect on the thyroid of the guinea pig. In this case again 44.5 mg. of guinea pig anterior pituitary is not more and is perhaps even less active than 3 mg. rat and 12 mg. rabbit anterior pituitary. In 4 out of 5 cases in which we inoculated 12 mg. rat anterior pituitary the hypertrophy of the thyroid gland of the guinea pig was very much more pronounced than the hypertrophy produced by similar weights of guinea pig or even of rabbit anterior pituitaries. This relative ineffectiveness of guinea pig anterior pituitary is perhaps related to the relatively low average size of the acinus cells of the thyroid gland or to the absence of interstitial gland in the ovary of the guinea pig. On the other hand, the thyroid gland of the guinea pig seems to be functionally very active to judge from the relatively large amount of organic iodine found in the blood of normal animals of this species (Closs, Loeb and MacKay[2]).

6253

Effect of Adrenal Cortex Extract on Maxillary Sinusitis in the Rabbit.

W. F. WENNER.

From the Department of Oto-Laryngology, Oscar Johnson Institute, Washington University.

Several investigators[1, 2, 3] have demonstrated that adrenalectomized animals showed a decreased resistance to infection and bacterial toxins. Blanchard[4] found that the opsonic power of the serum of adrenalectomized cats is greatly decreased. Phagocytic activity is reduced approximately 50% during the later stages of adrenal insufficiency. Sufficient work has been presented to show clearly the effectiveness of adrenal cortex extract in protecting against bacterial intoxication. Scott and Bradford[5] and Hartman and Scott[6] showed that adrenal cortex extract decreased the susceptibility of adrenalectomized rats to killed bacteria. Whitehead and Smith[7] indicate that cortical extract exerts some power in increasing the resistance of the human body to bacterial intoxication. Experiments reported here show that this extract causes infection of the maxillary sinus of rabbits to subside.

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[2] Closs, Karl, Loeb, Leo, and MacKay, Eaton, Proc. Soc. Exp. Biol. and Med., 1931, **29**, 170.

[1] Oppenheim and Loeper, *Compt. Rendu. Soc. Biol.*, 1903, **55**, 332.

[2] Jaffe and Marine, Proc. Soc. Exp. Biol. and Med., 1923, **21**, 64.

[3] Scott, *J. Exp. Med.*, 1924, **39**, 457.

[4] Blanchard, *Physiol. Zool.*, 1931, **4**, 302.

A series of 19 rabbits were given maxillary sinus irrigations with 0.5% solution of sodium alizarin sulphonate every 48 hours for 10 days.[8] Sinusitis, as evidenced by purulent nasal discharge, developed within 10 days following the last irrigation. Three intramuscular injections of adrenal cortex extract made by Dr. R. L. Zwemer[9] were given over a period of one week. The dosage used was 0.5 cc. per kilo body weight. The maxillary sinuses examined grossly and histologically at this time showed no evidence of infection. In the control rabbits which received the same number of irrigations but no cortex extract, the sinuses were filled with pus and the mucous membrane was infiltrated with leucocytes. Both total and differential white blood cell counts made on normal and experimental rabbits at the same hour of the day varied from day to day. Taking this day to day variation into consideration, adrenal cortex extract produced no change in the total or differential count. Hemoglobin fell from an average of 11 gm. to about 8 gm. per 100 cc. of blood (Newcomer method). CO_2 content and pH of blood plasma increased except in those rabbits having initial high pH and CO_2. These findings on the increase in plasma CO_2 following intramuscular injections of adrenal cortex extract agree with those reported by Zwemer.[10]

I wish to thank Dr. R. L. Zwemer for the generous supply of adrenal cortex extract used in these experiments.

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[5] Scott and Bradford, Proc. Soc. Exp. Biol. and Med., 1931, **28**, 428.

[6] Hartman and Scott, Proc. Soc. Exp. Biol. and Med., 1931, **28**, 478.

[7] Whitehead and Smith, Proc. Soc. Exp. Biol. and Med., 1932, **29**, 672.

[8] Wenner, *J. Lab. and Clin. Med.*, 1931, **16**, 341.

[9] Zwemer, Agate and Schroeder, Proc. Soc. Exp. Biol. and Med., 1931, **28**, 721.

[10] Zwemer, *Endocrin.*, 1931, **15**, 382.

6254

Studies on Variability of B. diphtheriae.

LLOYD JONES. (Introduced by M. S. Fleisher.)

From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.

Typical cultures of virulent diphtheria bacilli grown in broth containing 10% specific antibacterial serum gave origin to distinct and rather stable variant types differing markedly from the parent strain in colony form, lacking in fermentative properties and in virulence and toxigenicity. Likewise, continued cultivation of typical cultures (at 37°C.) in broth containing 0.1% phenol, of 0.5% lithium chloride or 2.5% pancreatin as well as cultivation in K-medium (Kendall) diluted 1:3 with physiologic salt solution and cultivation in plain broth at 45°C., gave rise to the development of similar variant types.

In some instances transformation from the original characteristic colony type to the variant type occurred suddenly while in other instances certain variations or intermediate changes in colony form with loss of virulence were noted before the appearance of the extreme variant type. The intermediate types probably corresponded to those noted by other observers of bacterial dissociation in *B. diphtheriae* cultures.

The variant or 'V' colonies are smaller, less opaque and grow less rapidly on various culture media. In morphology the 'V' organisms are pleomorphic, exhibiting small coccoid or occasionally somewhat elongated solid staining types. Broth cultures develop a uniform turbidity which is slow in appearing. The 'V' cultures are practically devoid of fermentative ability. Guinea pigs are not affected by inoculation with living 'V' cultures or with broth filtrates, indicating loss in virulence and in toxigenicity incident to the forced transformation.

These properties, which characterize the 'V' type culture have remained constant in cultures maintained over periods of 6 months to 2 years indicating considerable stability of the variant type.

Antisera containing agglutinins for the mother-type organisms were devoid of agglutinating effect upon some of the 'V' strains while other 'V' strains were agglutinated, indicating that agglutinogen common to the parent organism might be variably transmitted to the variant. In many instances variants apparently were dissimilar antigenically since antisera prepared against a particular variant frequently failed to agglutinate to any considerable extent other variants derived from the same parent organism.

Since there was no evidence that the variant type would spontaneously revert to the parent type organism an attempt was made to determine whether or not reversion from variant to original colony type, etc., could be induced by certain types of *in vitro* and *in vivo* cultivation, as has been accomplished with the rough variants of pneumococci, paratyphoid bacilli and other organisms. Accordingly the following technics were employed: *a*. variant cultures were transplanted in nutrient broth at 24-hour intervals over periods of 20 days, *b*. cultures were made in broth containing heat-killed organisms of the respective original type, *c*. cultures were made in broth containing heat-killed organisms of the parent type plus 5% of the 'V' antiserum, *d*. living 'V' type organisms were injected into guinea pigs, intraperitoneally, with heat-killed organisms of the original type. Cultural and pathogenic properties were not changed incident to these modes of cultivation and reversion in colonial form occurred only in one instance, wherein, one of the variants was injected into a guinea pig with heat-killed organisms of the original type and plates inoculated after 24 hours with the peritoneal fluid showed an admixture of parent-type with variant-type colonies. The former were lacking in pathogenicity and were quite unstable, readily dissociating into the 'V' type upon subculturing.

6255

Production of an Unsaturated Compound by B. coli in a Synthetic Medium.

W. F. LANGE. (Introduced by M. S. Fleisher.)

From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.

Production of unsaturated acids by bacteria in synthetic media has apparently rarely been observed. When *B. coli* were grown in a media* of known composition, it was noted that acidity continued

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* Synthetic Media 22.	gm.		gm.
Dextrin	10.0	Calcium Chloride	0.1
Potassium Hydrogen Phosphate	1.0	Ammonium Tartrate	10.0
Potassium Di-hydrogen Phosphate	1.0	Distilled Water	1000 cc.
Magnesium Sulphate	0.1		

pH, 8.6. Sterilized at 10 lb. for 15 minutes.

to increase even when tests showed the absence of lactic, acetic and aceto-acetic acids, and it was noted that the media readily absorbed bromine and iodine and decolorized potassium permanganate.

Cultures were made in large quantities of the media and allowed to grow for a period of 20 days. If tests for dextrin, reducing sugars, lactic acid ammonia, nitrites and nitrates were negative, the test for tartrate ion (alpha-beta-hydroxy-acid)† was less marked than in the control media and the pH was 5.0 or lower, the media was freed of the organisms by filtration through a Seitz filter. The filtrate was extracted 2 or 3 times with ether (free from peroxides). The ether was distilled from the extract. The residue, a water-clear liquid, was dissolved in a small quantity of water and reextracted with ether. The ether from the second extraction was removed by spontaneous evaporation in the dark, since light seemed to cause polymerization of the residue.

The residue, a water-like, colorless liquid, as stated above had the following qualities:

A strong acetic odor; readily soluble in water, ether, alcohol, and chloroform, a melting point of about 6°C. It was readily decomposed by sunlight but more slowly by diffuse light. Some of the extract was placed in a small vial, was kept in the icebox, but even under these conditions the material decomposed within 3 days.

It was found that 0.08 cc. of the liquid when dissolved in 3 cc. of 10% sodium carbonate solution decolorized more than 8 cc. of a 1% solution of potassium permanganate leaving a brown precipitate of manganese oxides; 0.13 gm. of the liquid dissolved in 2 cc. of carbon tetrachloride to which was added 3 drops of brom-solution (2 cc. of bromine to 50 cc. carbon tetrachloride) caused the decomposition of the bromine solution instantaneously without the evolution of hydrobromic acid. The decolorization of the brom-solution continued until 8 cc. had been added. When the solution was warmed an additional 2 cc. of the brom-solution was decolorized.

The decolorization of potassium permanganate and the absorption of bromine show that the residue is an unsaturated compound which may contain one or 2 unsaturated bonds. The large amounts absorbed seem to indicate that the compound has two unsaturated bonds.

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† The test here used was the formation of the copper complex ion, which is not specific for tartrate ion but may be obtained by any alpha-beta-hydroxy-acid. The product isolated from the media responds to this test and in all probability was responsible for the positive results.

The reaction with heavy metals is usually regarded as specific for propiolic acid when the odor, solubility, melting point, instability, and unsaturation have been established. A greenish yellow precipitate was formed at once when 1 cc. of a standard ammonical cuprous chloride solution was diluted with 5 cc. of distilled water and added to a drop of the ether extract. A whitish-yellow precipitate was formed which turned dark within a few seconds and left a mirror-like deposit in the test tube when silver nitrate was added to the aqueous solution.

The number of hydrogen atoms replaced by silver in this reaction is problematic. The instability of this compound makes it impossible to obtain accurate results. However, the summation of numerous determinations showed approximate results which indicated that one hydrogen atom of the ether extract was replaced by one silver atom after which decomposition occurred, leaving the mirror-like deposit. This unsaturated compound does not appear in the media in large quantities. It requires 12 liters of the media to obtain 1.06 gm. or 0.088 gm. per liter.

The product extracted from the media is very much like propiolic acid.[1] The purity of the material tested may be questioned in view of the limited attempts made to purify the substance. It is, however, very unstable and could be obtained only in such small quantities as to make further chemical analysis very difficult. Yet all tests applied to the substance clearly and distinctly indicate a pure product. The assumption that this acid is propiolic is based on the results obtained when tested as described by Mulliken.

It cannot be definitely stated that this acid is produced in the metabolism of the carbohydrates since during the lengthy period of growth some of the bacteria may have disintegrated and might thus be responsible for this product. If this were true the amount of acid should increase with time; however the best yields were obtained soon after tests for dextrin and the tartrate ion became negative and thereafter the substance gradually disappeared from media (25-30 days after inoculation).

When ammonium chloride is substituted for ammonium tartrate in the media, an alpha-beta-hydroxy-acid appears in the media between the 8-13 days and then disappears. This acid when extracted with ether responds to the same tests as that isolated when tartrate is used instead of chloride.

While it cannot be definitely stated yet it seems rather conclusive that this unsaturated acid is an intermediate product in the metabolism of dextrin.

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[1] Mulliken, Identification of Pure Organic Compounds, 1920, 75. Beilstein, *Handbuch der Organ. Chem.*, 1920, **2**, 477.

The product isolated has been shown to be an unsaturated acid which in every way corresponds to propiolic acid.

6256

Reversibility of Specific Adsorption of Bacterial Agglutinin.

MALCOLM H. MERRILL. (Introduced by Moyer S. Fleisher.)

From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.

The adsorption of agglutinin by specific organisms in the presence of low concentrations of electrolytes is a reversible reaction. This has been shown by the following experiment: Immune serum was diluted 1 to 50 with distilled water and added to distilled water suspensions of *B. coli* throughout a pH range of 2.5 to 10.5. Maximum adsorption as determined by titration of the supernatant after centrifuging occurs at pH 5.8 to 6.0, the adsorption becoming progressively less with either increasing acidity or alkalinity. At pH 3.0 and 10.5 adsorption was always less than 5% compared to 98 to 99% at pH 6.0. Washed organisms were used for adsorption and for the agglutinin determination.

When adsorption occurred at pH 6.0 and the sedimented bacteria were resuspended in distilled water at various pH values a reversal of adsorption occurred in both acid and alkali. The titer of these extracts was always distinctly lower than was the residual titer of the supernatant following adsorption at the same pH. If, however, the eluted extracts were mixed with the supernatant obtained after adsorption at pH 6.0, from which over 98% of the agglutinin activity had been adsorbed, the titer was now restored to that of the supernatant after simple adsorption at the corresponding pH. The addition of normal rabbit serum to the extracts to give the same content of normal serum constituents exerted the same effect.

The work demonstrates that reversal of adsorption is possible and indicates the dependence of agglutination upon the presence of normal serum constituents. This last factor appears to have been neglected by previous workers who have studied the reversibility of agglutinin adsorption.

6257

Components of the Vagus Nerve.

RUSSELL L. JONES. (Introduced by A. Kuntz.)

From the Department of Micro-Anatomy, St. Louis University.

The number of unmyelinated fibers in the cervical vagus trunk greatly exceeds the number of myelinated fibers. Kiss[1] regards the unmyelinated fibers as sympathetic fibers which join the vagus trunk mainly in the region of the superior cervical sympathetic ganglion. He denies the existence of unmyelinated fibers in the vagus rootlets. According to Ranson and Chase,[2] and others, the unmyelinated fibers in the vagus trunk arise from cells in the brain stem and emerge in the vagus rootlets.

In the present investigation, carried out on cats, anatomical and experimental studies were made of the relationships between the cervical sympathetic and vagus trunks and their myelinated and unmyelinated components.

The superior cervical sympathetic and nodose ganglia were removed together from several cats. Threads were then tied to each of the ends of the respective trunks and the 2 ganglia were tied upon a glass frame with sufficient tension to draw them slightly apart, to provide an opportunity for the demonstration of any possible connections between them when the preparation was later sectioned. In several instances the cervical vago-sympathetic trunk was removed and sectioned transversely at intervals of less than 0.5 cm. Some of these preparations were stained with osmic acid and some with pyridine silver.

Study of the sections showed that the 2 ganglia are connected by several small rami which contain both myelinated and unmyelinated fibers. These small rami contribute no more than a small fraction of the unmyelinated fibers of the vagus trunk. Sections of the vago-sympathetic trunk taken at short intervals show no interconnecting branches between the vagus and sympathetic trunks distal to the nodose ganglion.

The vagus trunk is joined by rami from the inferior cervical sympathetic ganglion. These are more apparent on the right than on the left side. Schinozaki[3] also finds this to be true in man. The sum total of all the fibers which join the vagus from the inferior cervical sympathetic ganglion is relatively small as compared with the total numbers of fibers in the vagus trunk.

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[1] Kiss, F., *Arch. du Museum National d'Historie Naturelle*, 1931, **6**, 7.

[2] Ranson, S. W., and Chase, M., *J. Comp. Neur.*, 1914, **24**, 31.

[3] Schinozaki, S., *Folio Anatomica Japonica*, **6**, 599.

Eight cats were subjected to extirpation of the superior and inferior cervical sympathetic ganglia on the same side. In 4 the ganglia were removed from the right, and in 4 from the left side. The inferior ganglion usually was removed first and the superior ganglion 4 to 7 days later, and the cats were autopsied 10 to 21 days later. Portions of both vagi were taken at various levels, 1 to 2 cm. above the origin of the recurrent nerve, 1 to 2 cm. below this point, and 1 to 2 cm. below the heart. The tissues from 4 cats were stained with osmic acid and those from the other 4 with pyridine silver. Sections of the vagus on the operated side were compared with those on the unoperated side.

The operations had but little effect on the vagus trunk at either of the 3 levels described. Pyridine silver sections of the vagus nerve of 2 cats just distal to the recurrent nerve show a very small area of degeneration on the operated side. This probably represents a small contribution of fibers from the inferior cervical sympathetic ganglion.

Various workers have called attention to an apparent increase in the number of unmyelinated fibers in the vagus trunk in the region of the nodose ganglion. The results of recent work indicate that there is no increase in the number of unmyelinated fibers in the trunk below the nodose ganglion, *i. e.,* myelinated fibers do not lose their myelin sheaths as they are traced distally. Careful study of sections of the vagus rootlets reveals unmyelinated fibers in considerable abundance, but in much smaller numbers than the numbers of unmyelinated fibers present in the vagus trunk distal to the nodose ganglion. The vagus trunk proximal to the nodose ganglion contains unmyelinated fibers in approximately the same numbers as the vagus rootlets.

Comparison of the vagus trunk proximal and distal to the nodose ganglion shows a great difference in the ratio of myelinated to unmyelinated fibers. Proximal to the ganglion the greater part of the cross-sectional area of the trunk is occupied by myelinated fibers, while distal to it the greater part of the cross-sectional area is occupied by unmyelinated fibers. Serial sections through the nodose ganglion show that this change is effected by a gradual increase downwards through the ganglion in the number of unmyelinated fibers. Another factor in the change is the deviation from the vagus trunk of the fibers which make up the superior laryngeal nerve. Counts made of the fibers in the vagus trunk proximal and distal to the nodose ganglion and in the superior laryngeal nerve are recorded in Table I. These counts indicate quite clearly that the nodose ganglion is the site of origin of the majority of the unmyelinated fibers in the vagus trunk.

TABLE I.

	Cat No. 40, Osmic Acid No. myelinated fibers	Cat No. 25, Pyridine Silver No. unmyelinated and myelinated fibers
Left vagus above nodose	5,377	7,877
Left superior laryngeal	2,182	2,808
Left vagus below nodose	2,848	11,872

The large size of the nodose ganglion, as expressed especially by its length, might indicate that it probably is more than an ordinary sensory ganglion. If the cells in the nodose ganglion were associated only with afferent fibers, there should be fewer ganglion cells in this ganglion than there are fibers above it. Counts of the cells in the nodose ganglion and the myelinated fibers in the vagus trunk proximal to the ganglion are recorded in Table II.

TABLE II.
Cat No. 34—Osmic Acid.

No. of cells in nodose ganglion	14,384
Myelinated fibers in vagus trunk above ganglion	4,263

The number of unmyelinated fibers in this trunk could not be determined since the stain used was osmic acid. The data of Table I, which were obtained from a cat of the same size, however, show that the number of cells in the nodose ganglion far exceeds the total number of myelinated and unmyelinated fibers in the vagus trunk proximal to the ganglion. The number of cells counted closely approximates the number of fibers counted in the vagus trunk distal to the ganglion. The greater number of the cells of the nodose ganglion, therefore, must be regarded as the cells of origin of the unmyelinated fibers.

The vagus trunk was severed above the nodose ganglion on the right side in one cat, and on the left side in another. After a degeneration period of 15 days, these cats were autopsied and pieces of the vagus nerves distal to the ganglia were obtained and prepared for study. These preparations showed only partial degeneration of the unmyelinated fibers, indicating that the major portion of the unmyelinated fibers arise from cells in the nodose ganglion. The degenerated fibers in these preparations presumably represent preganglionic components of the vagus which terminate in ganglia located farther distally. These results confirm the physiological evidence of the existence of efferent cells in the nodose ganglion recently reported by Morgan and Goland.[4]

6258

Estimation of Hemoglobin on a Basis of Protein Iron.

A. P. BRIGGS.

From the Department of Internal Medicine, St. Louis University Medical School.

There have been several micro-methods in the recent literature for the determination of total iron in blood,[1, 2, 3] and it has been suggested that these determinations be used for the calculation of hemoglobin. On account of the constant breakdown of hemoglobin to bilirubin there is a variable quantity of liberated inorganic iron which may amount in certain anemias to more than 5% of the hemoglobin iron.[4] Consequently it would seem advisable to make a preliminary separation of inorganic iron from hemoglobin. Riecker[4] has observed that such a separation may be accomplished by means of a trichloracetic acid precipitation.

In the present study hemoglobin has been calculated from the iron determined in the trichloracetic precipitate from whole blood according to the following technique: A 1 cc. sample of blood is transferred to a 50 cc. centrifuge tube, baked with N/10 HCl and precipitated with trichloracetic acid. After centrifugation the supernatant fluid is poured off, the precipitate dissolved in a minimal quantity of NaOH, transferred to an Erlenmeyer flask, and digested with 5 cc. of nitric acid and 0.5 cc. of 60% perchloric acid. The digest is dissolved in 5 N H_2SO_4, transferred to a 50 cc. volumetric flask and the prussian blue color developed, using gum ghatti as a protective colloid. A standard of known iron content for comparison is prepared at the same time. This technique for determination of protein iron has been used to standardize a dry hemoglobin preparation which is used for the determination of hemoglobin by means of the acid hematin color, somewhat according to the plan of Elvehjem[5] and Arnow.[6]

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[4] Morgan, L. O., and Goland, P. P., *Anat. Rec.*, 1932, **52**, 26.

[1] Wong, S. Y., *J. Biol. Chem.*, 1923, **55**, 421.

[2] Kennedy, R. P., *J. Biol. Chem.*, 1927, **74**, 385.

[3] Reis, Frederick, and Chakmakjian, H. H., *J. Biol. Chem.*, 1931, **92**, 59.

[4] Riecker, Herman H., and Winters, Mary E., *Am. J. Physiol.*, 1930, **92**, 197.

The hemoglobin powder is prepared from blood by precipitating the hemoglobin and other proteins with NaCl at saturation after acidification with hydrochloric acid. The precipitate is washed with strong NaCl solution, dried and pulverized. In use a weighed quantity of the powder is dissolved in N/10 NaOH and transferred to a volumetric flask. In another flask the test sample of blood is treated similarly with NaOH. After a few minutes the contents of both flasks are diluted to the mark with water and sufficient HCl to make the final concentration N/10.

TABLE I.
Hemoglobin by Protein Fe and O_2 Capacity.

Subject	Basis of Protein Fe Direct as Prussian blue	Basis of Protein Fe Indirect as acid hematin	Basis of O_2 Capacity
B. P.	17.35	17.10	17.00
W. L. N.	17.09	16.70	16.58
J. G.	18.11	18.05	18.00
Pernicious Anemia	5.28	5.32	4.56*
Leukemia	9.82	9.72	9.72
Malaria	9.37	9.26	9.68
Pneumonia	13.41	13.32	13.85
Jaundice	14.15	14.26	14.13
Nutritional Edema	12.00	11.82	12.15

* Low result due to poor quality saponin.

In the accompanying table are data on three normal individuals and a few patients, showing close agreement between hemoglobin values calculated from oxygen capacity and from protein iron. Figures represent gm. percent.

6259

Effect of Theelin Injections upon the Castrated Woman.

AUGUST A. WERNER. (Histological Study by W. D. Collier.)

From the Departments of Internal Medicine and Pathology, St. Louis University School of Medicine.

These experiments were an attempt to determine the effect of various dosages of theelin upon human female castrates of different ages and for varying periods of castration with particular interest in the effect upon the subjective symptoms of castration and the involuntary changes of the breasts and genital tract.

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[5] Elvehjem, C. A., *J. Biol. Chem.*, 1931, **93**, 203.

[6] Arnow, L. Earl, Proc. Soc. Exp. Biol. and Med., 1932, **29**, 569.

Five white women who had complete bilateral ovariectomy performed and whose uteri were intact were used in these experiments. The ages ranged from 22 to 40 years. The age at castration ranged from 21 to 38 years and the time elapsing since castration ranged from 6½ months to 6 years. All had amenorrhea and the subjective symptoms of castration and all showed atrophic breasts and genital tracts upon physical examination. Curretage of the endometrium showed extensive atrophy of the endometrium in all cases. All other physical and laboratory tests were normal.

All patients received daily a dose of 200 rat units of theelin intramuscularly in the upper genital region during the first 28 days, 300 rat units during the second 28 days, and 400 rat units during the third 28 day period. After the third period, injections were stopped to determine if uterine bleeding would occur as claimed by some writers. The total period of daily observation of each patient was over 5 months.

All patients showed relief from subjective symptoms of castration in from 6 to 20 days. All showed an increased congestion of the cervix and a mucous discharge from the cervix within the first two weeks. All patients showed enlargement of the breasts, erection of the nipples and sometimes even complained of the tingling and sense of fullness of the breasts. The oldest patient, 40 years of age, alone failed to show a relatively marked hyperplasia of the endometrium and alone failed to show bleeding from the uterus accompanied by all the usual subjective symptoms of menstruation. Uterine bleeding occurred in 4 patients during the administration of theelin and 3 showed bleeding during the first week after the hormone was discontinued. The endometrium was not only increased in thickness in all except the one patient mentioned but it also became much more differentiated. The mucoid type of stroma found at the beginning eventually differentiated out normal hyperchromatic stroma cells and the epithelium differentiated out more fully, sending down longer and more tortuous tubules.

Obviously excessive doses of theelin were given over a period of at least 84 days without discomfort until 6 to 8 cc. were given, and then there was only local and transitory discomfort.

6260

Isolation and Cultivation of Bacterium melaninogenicum.

KENNETH L. BURDON. (Introduced by J. Bronfenbrenner.)

From the Department of Bacteriology and Immunology, Washington University School of Medicine, St. Louis.

We previously pointed out[1] the constant occurrence of *Bacterium melaninogenicum*[2] in the normal mouth and on the external genitalia, and its abundance in connection with various pathologic processes.

Since then, our experience and that of others has amply confirmed the very frequent association of this bacterium with infections of various types, and while there is no satisfactory evidence that the organism is by itself invasive, it is one of the most prominent concomitant organisms in pyorrhea, lung abscess, sub-acute puerperal fever, and similar mixed infections.

Workers have not yet succeeded in maintaining the organism in pure culture, and therefore its systematic description and identification have not been attempted. The organism will develop readily for a time in symbiosis with other organisms (such as *Strep. viridans*), but its ultimate isolation from such mixtures is no easier than from the primary cultures, and it is soon lost.

Recognizing that the formation of pigment by an organism is associated with senescence, Dr. Bronfenbrenner suggested that the almost constant failure to secure pure growth in transfers from pigmented colonies is probably due to loss of viability of the organisms. It was found that this assumption is correct, and that pure cultures can be secured and maintained if transfers are made very frequently from colonies before they develop much pigment. The original material is streaked directly upon plates of freshly made hormone blood agar (pH 7.4) and the plates are incubated for about 4 days in an anaerobic jar. Single colonies which are just beginning to show a brownish color are then fished to warmed plates of the same medium. Thereafter the pure growths must be transplanted at short intervals (48-72 hours) until they become adjusted to artificial conditions. Cultures usually are not viable for longer than 6 or 7 days, except after several months of cultivation. The following are among the principal properties of the several strains which have been studied.

The organism is a Gram negative, non-spore-bearing, non-motile, anaerobic bacillus, showing considerable variation in morphology from a very small cocco-bacillus to longer rods, and occasional thread forms. The rods are frequently vacuolated and show bipolar staining. There is a marked tendency for the organism to undergo spontaneous autolysis after a few days growth, particularly in liquid media. On blood agar the organisms produce hemolysis which varies in intensity with the amount of the pigment, and is slight when cultures are transplanted frequently on this medium. The organism does not ferment dextrose, nor any other of the 8 common carbohydrates tested. It is strongly proteolytic, causing digestion of coagulated serum, egg, and milk, and to a less extent the proteolysis of meat and brain media, with the slow formation of gas. Gelatin is liquefied. A large amount of indol is produced.

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[1] Burdon, K. L., *J. Infect. Dis.*, 1928, **42**, 161.

[2] Oliver, W. W., and Wherry, W. B., *J. Infect. Dis.*, 1921, **28**, 341.

The characteristic pigment is formed only in media containing hemoglobin. The pigment appears late, after active multiplication has ceased. It always develops first in the center of single colonies, in the immediate vicinity of a contaminating colony (where growth is accelerated) and, in pure cultures, in the areas of heaviest growth. The pigment can be extracted from the bacteria in acidified methyl alcohol, and precipitated by neutralization. The amorphous brownish-black powder thus secured is soluble in dilute alkali, but insoluble in water, ether, chloroform, neutral methyl or ethyl alcohol, xylol, dilute acids, carbon disulphide, or carbon tetrachloride.

The organism is not virulent when tested by intraperitoneal and intravenous injections of actively growing pure cultures into guinea pigs and rabbits. One strain, however, has produced an extensive cutaneous gangrene in these animals when inoculated subcutaneously, with death of the animals in about 48 hours. There were no evidences of septicemia.

6261

Effect of KI upon Basal Metabolism and Respiratory Quotient in Thyroidectomized Guinea Pigs.*

WALTER J. SIEBERT.

From the Department of Pathology, Washington University School of Medicine.

Loeb,[1] Gray and Rabinovitch,[2] McCordock,[3] and others have found that the administration of potassium iodide causes a marked stimulation of the thyroid gland in guinea pigs, as shown by a great increase in the number of mitotic figures, a slight softening of the colloid and an increase in the height of the acinar epithelial cells. Cordonnier[4] and the author[5] found that the basal metabolism was only very slightly affected after administration of KI in guinea pigs. In the latter experiments, the majority of the determinations as well as the average for all the animals was at the upper limit of the variations obtained in normal animals. Loeb[1] suggested the following possibilities to explain this discrepancy which exists between the marked degree of stimulation of the thyroid gland and the very slight effect upon basal metabolism after administration of KI. (1) Although KI stimulates the thyroid gland at certain periods of its action at the same time it prevents an excess of thyroxin thus produced from leaving the acini, and instead it is at least partly retained in the gland. (2) The stimulation of the acini does not necessarily lead to an excess production of thyroxin. (3) Peripheral effects counteract the stimulating effect of the thyroid hormone upon the basal metabolism.

The following experiments were carried out to investigate the peripheral action of KI. Twelve male guinea pigs, weighing between 400 and 450 gm., were as completely thyroidectomized as was possible, and 3 weeks after the operation, 8 were given 0.05 gm. of KI by mouth daily and biweekly metabolism tests were made. The remaining 4 guinea pigs served as controls. These were similarly thyroidectomized but were not given KI. At the

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* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Loeb, Leo, *Am. J. Path.*, 1926, **2**, 19.

[2] Gray, S. H., and Rabinovitch, J., Proc. Soc. Exp. Biol. and Med., 1929, **26**, 468.

[3] McCordock, Howard A., Proc. Soc. Exp. Biol. and Med., 1928, **26**, 109.

[4] Cordonnier, J., Proc. Soc. Exp. Biol. and Med., 1929, **26**, 636.

[5] Siebert, Walter J., and Smith, Robt. S., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 629.

operation, a careful effort was made to remove all of the thyroid gland tissue and to leave behind sufficient parathyroid tissue to prevent tetany from occurring. It was found, however, when autopsies were performed and preparations made of suspicious tissue for microscopic confirmation that in the majority of animals small remnants of thyroid tissue could be found. It is very probable that these remnants of thyroid gland tissue become sufficiently large to be seen only after compensatory hypertrophy has occurred. The control animals and the KI fed animals were tested at the same time under similar conditions. The Haldane open circuit respiratory calorimeter was used to make the tests, made after a 24-hour fasting period. Amytal (Lilly) in small doses was injected subcutaneously just prior to the trial periods to keep the animals quiet while determinations were being made. Two hour test periods were used in each case. The basal metabolism values of the 4 control animals ranged from 2.0 to 3.14 calories per kilo per hour; while the KI fed animals showed variations between 1.91 and 3.42 calories per kilo per hour before the administration of KI began. Thus the values for the basal metabolism in these guinea pigs after complete or almost complete thyroidectomy ranged between 0 and 45% below the average for normal guinea pigs. Within a few days following the daily administration of 0.05 gm. KI by mouth all 8 guinea pigs began to show basal metabolism values distinctly lower than those obtained in the animals which were thyroidectomized but to which no KI was administered. This lowering of the basal metabolic rate continued throughout the entire period of the experiments, from 14 to 30 days. The values obtained in the KI fed animals ranged between 1.52 and 3.30 calories per kilo per hour; and in all except a very few determinations the rates were distinctly lower in each individual experiment in this group than in those obtained at the same time in the control animals. This alteration in the basal metabolic rate ranged from 2% above to 55% below the average of those which were thyroidectomized but to which no KI was administered. The total average of the 36 determinations for the control group was 2.87 calories per kilo per hour, which is 18% below the average which we obtained in normal guinea pigs; while the total average for the 50 determinations of those which had received KI was 2.38, 32% below that of normal animals. Thus the average basal metabolism was approximately 14% lower in the 8 thyroidectomized guinea pigs which had received KI than the average of the 4 thyroidectomized guinea pigs to which no KI was administered.

The respiratory quotients of the thyroidectomized guinea pigs which had received KI were distinctly higher in the large majority of cases as compared with those which did not receive KI. The respiratory quotients varied between 0.706 and 0.819 in the control group, whereas in the KI fed group they ranged between 0.727 and 1.04. The total average of all the respiratory quotients in the controls was 0.747, as compared with 0.859 in the KI fed group. In only 12 out of 50 determinations were the respiratory quotients within the range of the control, while in the remaining 38 tests, the KI fed guinea pigs had respiratory quotients distinctly higher than those of the control experiments. There was no correlation between the lowering of the basal metabolic rate and the rise in the respiratory quotient in individual cases.

Conclusions. The administration of KI to completely or almost completely thyroidectomized guinea pigs causes (1) a lowering of the basal metabolic rate and (2) a rise in the respiratory quotient. The peripheral effect of KI in causing a depression in basal metabolism is at least partly responsible for the fact that KI causes only a very slight effect upon the basal metabolism although the thyroid gland shows evidence of a marked degree of stimulation.

Peiping Section.

Peiping University Medical College, April 28, 1932.

6262

A Comparative Study of Various Typhoid O Antigens.

D. HUIE WONG AND S. H. ZIA. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, and Department of Medicine, Peiping Union Medical College.

In view of the increasing interest bacteriologists and clinicians have taken in 0 agglutination in typhoid fever, and the different results[1, 2, 3, 4, 5] reported with the use of various antigens, it seems worth while to report a comparative study of 3 commonly used 0 antigens in human cases.

Two of the suspensions were prepared from a smooth Rawlings strain of *B. typhosus* culture, one of which was treated with alcohol according to Gardner's modification of Bien's method;[1,] * the other was cultured in 0.1% phenol broth (Braun[6]). The third suspension was a formalized suspension of Ty 901 strain (a non-motile *B. typhosus*) kindly supplied to us by Gardner. The 3 suspensions were not used throughout the study; alcohol and phenol suspensions were used in the first part of the work and alcohol and Ty 901 suspensions in the latter part of the work.

Dreyer's macroscopic agglutination technique was followed in all tests. Incubation was at 56°C. over night. The titre was considered

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[1] Gardner, A. D., *J. Hyg.*, 1929, **28**, 376, and personal communication.

[2] Felix, A., *J. Hyg.*, 1929, **28**, 418.

[3] Whitehead, N. T., *J. R. A. M. C.*, 1930, **55**, 81.

[4] Stuart, G., and Kukorian, K. S., *J. Hyg.*, 1929, **28**, 105.

[5] Pijper, A., *J. Hyg.*, 1929, **29**, 380.

* Suspend an 18-24 hour growth in a small amount of 0.5% carbolized physiological saline. Add an equal volume of absolute alcohol. Stand overnight at 37°C. Add saline to reduce the alcohol concentration to 33%. Shake well and leave standing in a cylinder for a few hours to allow foreign matter, clumps, etc., to deposit. Pour or pipette off the supernatant suspension which is used in the tests.

[6] Braun, H., *Berl. klin. Wchnschr.*, 1918, **55**, 637.

as the highest dilution in which a 2 plus agglutination occurred. All tests resulting in a positive reaction gave a typical granular agglutination. In reporting results only those cases having a titre of 1:160 or above were considered positive.

The results were tabulated into 3 main groups (1) typhoid cases; (2) cases vaccinated with T.A.B., subdivided according to the route of inoculation, subcutaneous or intravenous, since one of us[7] found that this affects the incitement of the 0 agglutinins, (3) all other cases which served as control. Table I gives the results obtained with 427 cases, 239 of which were tested with the alcohol and phenol suspensions and 188 of which were tested with the alcohol and Ty 901. The 3 suspensions did not act similarly in all cases. The Ty 901 suspension was the most specific, as it reacted positively only in cases of typhoid infection and those intravenously inoculated. The alcohol treated suspension gave a lower percentage of positive reactions in typhoid cases than either the Ty 901 or phenol. It was not as specific as Ty 901 as a few of the non-typhoid cases reacted positively with it, but was more specific than the phenol suspension which gave a higher positive percentage among the non-typhoid cases. The phenol suspension although the most sensitive as regards the disease itself, had the disadvantage of being least specific. These results may be an explanation of the conflicting reports in the literature as to the presence of 0 agglutinins in patients who have had previous subcutaneous inoculations with the T.A.B. vaccine, and it, therefore, seems best to use the Ty 901 antigen as a standard 0 suspension in the diagnosis of typhoid fever.

TABLE I.
Specificity of Various O Antigens.

	Typhoid Cases			Vaccinated Cases						Control Cases			Total Cases
				Subcutaneous route			Intravenous route						
Antigen	Total	Pos.	%	Total	Pos.	%	Total	Pos.	%	Total	Pos.	%	
Alcohol	33	17	52	13	1	8	6	2	33	136	2	1	188
Ty 901		21	63		0	0		5	83		0	0	
Alcohol	24	18	75	69	1	1	6	4	67	140	3	2	239
Phenol		21	87		5	7		5	83		5	4	

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[7] Zia, S. H., Proc. Soc. Exp. Biol. and Med., 1931, **29**, 253.

6263

Active Bacterial Anaphylaxis in Relation to Antibody Production.

C. E. LIM AND T. J. KUROTCHKIN.

From the Department of Bacteriology and Immunology, Peiping Union Medical College.

The conception of anaphylaxis as an antibody-antigen reaction is generally admitted but the existence of a special anaphylactic antibody has never been conclusively demonstrated. The experiment of Doerr and Russ[1] indicated that a very definite quantitative relationship between precipitin content of the blood and its sensitizing power existed. The hypothesis of the essential identity of anaphylactic antibody and precipitin remained unchallenged until further light was thrown upon the subject by subsequent investigations. Manwaring and others[2] have shown that in dogs the sensitizing potency of the serum is probably different from its immune power. Spain and Grove[3] have also found that precipitin and anaphylactic reactions in rat might possibly represent 2 different immunological phenomena. Our previous experiment[4] on active sensitization of guinea pigs with certain strains of the yeast-like fungi has also resulted in a finding indicating that the sensitizing power of closely related species of these organisms is distinctly different.

It seems of interest to determine whether a correlation between sensitizing and antigenic properties of different moniliae could be found. For the present experiment, *Monilia pinoyi* and *Monilia psilosis* have been selected. The biological difference between these 2 species consists mainly in the fact that the former fungus exhibits a considerably higher sensitizing power than the latter. Specific polysaccharids were prepared from each monilia strain, using a similar method. The serological tests performed with each polysaccharid showed that their specific activities in precipitin and complement fixation reactions when set up against homologous rabbit antiserum gave similar readings. In cross precipitin reactions both polysaccharids also showed identical titres.

Active sensitization of guinea pigs was performed through the use of 48 hour Sabouraud agar cultures suspended in normal saline to an equal density and killed by exposure to 75°C. for 45 minutes. A series of young and approximately equal weight guinea pigs was divided into 4 groups, each receiving 1 to 4 doses respectively, each dose consisting of 1 cc. of the respective monilia suspension, administered intraperitoneally at 3-day intervals. Two weeks after the last injection each animal was bled through heart puncture, following which it received the intravenous injection of a dose of the homologous polysaccharid dissolved in 1 cc. of saline. The blood collected was then tested for precipitin and complement fixing antibodies against various dilutions of the polysaccharid. The anaphy-

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[1] Doerr, R., and Russ, V. K., *Z. f. Immunitätsforsch.*, 1909, **3**, 181.

[2] Manwaring, W. H., Shumaker, P. W., Wright, P. W., Reeves, D. L., and Moy, H. B., *J. Immunol.*, 1927, **13**, 59.

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[4] Kurotchkin, T. J., and Lim, C. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 223.

TABLE I.
Antibody Content and Active Anaphylaxis of Guinea Pigs Sensitized with

Doses of Monilia Injected	Precipitin Titres	Complement Fixation Titres	Intravenous dose of Specific Carbohydrate in mg.	*Monilia pinoyi* Anaphylactic Response		*Monilia psilosis* Anaphylactic Response	
				Positive	Negative	Positive	Negative
1	—	—	4	—	2	—	2
			2	4	1	—	2
			1	1	4	—	3
2	—	—	2	2	—	—	1
			1.5	2	1	—	3
			1	5	—	—	3
			0.5	—	2	—	1
3	1:100- 1:10,000	1:250	2	2	—	2	—
			1.5	2	—	2	1
			1	4	1	3	—
			0.5	—	2	—	2
4	1:500- 1:10,000	1:250	4	—	2	—	2
			2	3	—	3	—
			1	3	—	2	—
			0.5	—	2	—	2

lactic response of each animal and the antibody content of its blood were recorded.

Results. As seen from Table I, the sensitization of guinea pigs with dead cultures of the *Monilia pinoyi* showed that irrespective of the number of the monilia injections, positive anaphylactic response has been secured in each group of animals. Fatal shock was generally produced in those animals which received 1-2 mg. injection of the polysaccharid. The determination of the precipitin and complement fixing antibodies in the blood of animals sensitized with the *Monilia pinoyi* showed that after 2 intraperitoneal injections of the fungous culture, no demonstrable antibodies were present. Positive reaction both in precipitin and complement fixation test occurred only in the blood of those animals which received at least 3 monilia injections. Positive precipitin reaction was observed with dilutions of the specific polysaccharid ranging from 1:100 to 1:10,000. On the other hand, the complement fixation test gave a considerably lower titre, being positive only with dilutions not exceeding 1:250 of the polysaccharid.

Sensitization of guinea pigs with the culture of the *Monilia psilosis* and subsequent determination of the antibody content in the blood of these animals gave results precisely similar to that found in animals sensitized with the *Monilia pinoyi.*

The anaphylactic response of animals sensitized with the *Monilia psilosis* was, however, quite different from that obtained from *Monilia pinoyi.* Animals receiving one or 2 injections of the monilia suspensions and showing no antibodies in their blood, responded to the injection of the polysaccharid negatively. Positive anaphylactic shock occurred in those animals which received more than 2 injections of the monilia culture and showed demonstrable antibodies in their blood.

Conclusion. In an attempt to determine the relationship between anaphylactic response and antibody content in guinea pigs actively sensitized with different strains of yeast-like fungi, it was found that in case of *Monilia pinoyi* sensitization appeared before the precipitin or complement fixing antibodies could be demonstrated. A similar experiment with the *Monilia psilosis* showed that anaphylactic response was produced in guinea pigs only in the presence of demonstrable precipitin. The experiment indicates also that, so far as guinea pigs are concerned, the reaction of precipitation is considerably more sensitive than that of complement-fixation. The findings presented here may suggest that in certain species of yeast-like fungi, the highly developed sensitizing power constitutes the differential biological characteristic of the fungus.

6264

Susceptibility of the Chinese Stripted Hamster (Cricetulus Grisens) to Treponema pallidum.

CH'UAN-K'UEI HU AND LOUISE PEARCE.

From the Peiping Union Medical College.

Experiments to determine whether the Chinese hamster is susceptible to inoculation with *Treponema pallidum* have shown that organisms pathogenic for the rabbit may be recovered from certain tissues of this animal species. It would appear that there is dissemination of the organisms from the local focus of inoculation and presumably a multiplication of them occurs.

Hamsters were injected in one testicle with a tissue (orchitis) emulsion rich in spirochetes obtained from rabbits which had been inoculated with either the Nichol's strain or with a strain (P III) isolated from a patient in this hospital in 1926. At varying periods, the hamsters were killed by ether anesthesia and different tissues, emulsified with sterile normal saline were injected intratesticularly in normal rabbits. The rabbits were under observations approximately 3 months. In the case of those in which a clinical orchitis was not detected together with darkfield demonstration of spirochetes, the inoculated testicle and the popliteal lymph nodes were injected in a second set of rabbits; in a few instances, this procedure was repeated in a third set of rabbits.

The available results on the first generation of rabbit transfers may be summarized as follows: (1) In rabbits injected with the inoculated hamster testicle, 37, 64, and 138 days previously, a syphilitic orchitis developed in 2 to 3 weeks. (2) The inguinal lymph nodes of a hamster which was inoculated 64 days previously, induced a syphilitic orchitis in 2 rabbits in 7 and 9 weeks respectively; nodes from a hamster inoculated 138 days previously gave negative results in one rabbit. (3) Brain tissue of a hamster injected 138 days previously, induced a syphilitic orchitis in a rabbit in 43 days; negative results followed the injection of brain tissue of 2 hamsters injected 37 to 64 days previously. (4) Negative results followed the injection of *uninoculated* hamster testicle (64 and 138 days) and of heart's blood (24, 37, 64, and 138 days).

No gross changes were detected in the organs of the hamsters and furthermore, no spirochetes were demonstrated by darkfield in any of the tissue emulsions examined.

The sojourn of the organisms in the hamster for one passage generation did not appear to affect the pathogenicity of the strains for the rabbit, as judged by the length of the incubation period of the orchitis and by the subsequent development of generalized lesions.

6265

Further Evidence of Additional Substances Essential to Mammalian Nutrition

H. K. STIEBELING (Introduced by H. C. Sherman)

From the Department of Chemistry, Columbia University

This paper briefly reports some of the experimental evidence encountered in the course of experiments on vitamin G, which can best be interpreted by postulating the existence of more than the 6 vitamins generally recognized as factors in mammalian nutrition. The evidence included is of two types. The first type deals with the supplementary nature of 2 sets of vitamin G-containing products, and the second with the contrasting growth response of animals of different nutritional history, during the latter part of a vitamin G-test period.

In testing by the Bourquin and Sherman method[1] the vitamin G potency of laboratory preparations obtained in fractionation and concentration experiments, a supplementary effect on growth was observed when a mixture of 2 fractions of skim milk powder was fed, as contrasted with the results obtained upon feeding twice the quantity of either fraction by itself. Fig. 1 shows the effect upon gain curves of feeding daily, (*a*) a definite quantity ($2x$) of an extract made at room temperature from skim milk powder with 80% (by weight) alcohol; (*b*) a definite quantity ($2y$) of the residue left after extracting the milk powder as described in (*a*), and (*c*) a mixture ($x + y$) of half of each quantity of the products just described. During the first few weeks of the test period, the rate of gain permitted by the mixture of the 2 supplementary materials was between that permitted by either given separately, as would be expected if vitamin G were the sole growth-limiting factor. But during the latter part of the test period, the rate of growth permitted by the mixture exceeded that allowed by either of the supplements alone. These results can be explained by postu-

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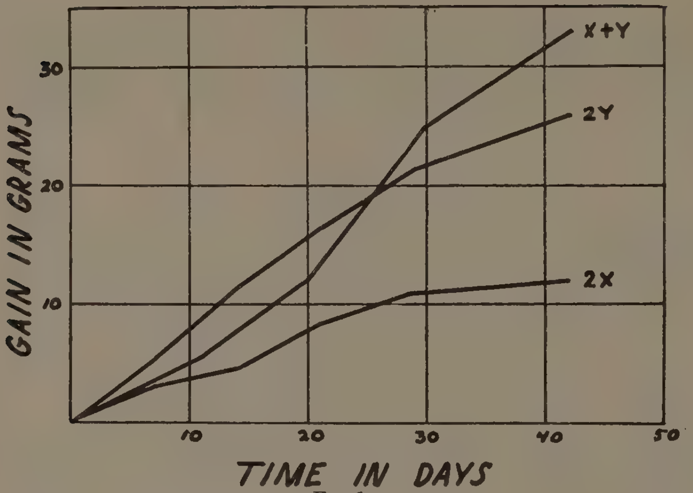

FIG. 1.

Gain curves of rats between the 6th and 12th weeks of their life, showing in vitamin G tests a supplementary effect of two products upon growth. X is an extract of skim milk powder made at room temperature with 80% alcohol; Y is the residue corresponding to X. Each curve represents averages of 3 or 4 experiments.

lating that the bodily requirement of some nutritive essential other than vitamin G (in the strict sense of the Bourquin method) was not furnished by the basal diet, and was unequally supplied by the 2 fractions of the milk powder, the residue containing relatively more of the "new" factor. This factor was furnished to the group of animals receiving the extract from a bodily store of the animals, until this reserve was depleted, whereupon growth practically ceased for lack of the "new" factor. On the other hand, the mixture of the 2 fractions contained enough of the "new" factor, or enough was furnished jointly by the bodily store and by the mixture fed, to permit continuous growth throughout the experimental period.

This view is confirmed by the data presented in Fig. 2. The gain curves there shown are of experimental animals which had received from one to 2 units of vitamin G between the 6th and 14th weeks of their life, the period usually included by a vitamin G test. At the end of this period the bodily store of the "new" factor was probably much depleted. Matched animals were then given (*a*) a definite quantity of an extract ($2x$) made from skim milk powder by treating it with 80% alcohol at room temperature; (*b*) a definite quantity of a filtrate ($2z$) left after treating with Lloyd's reagent

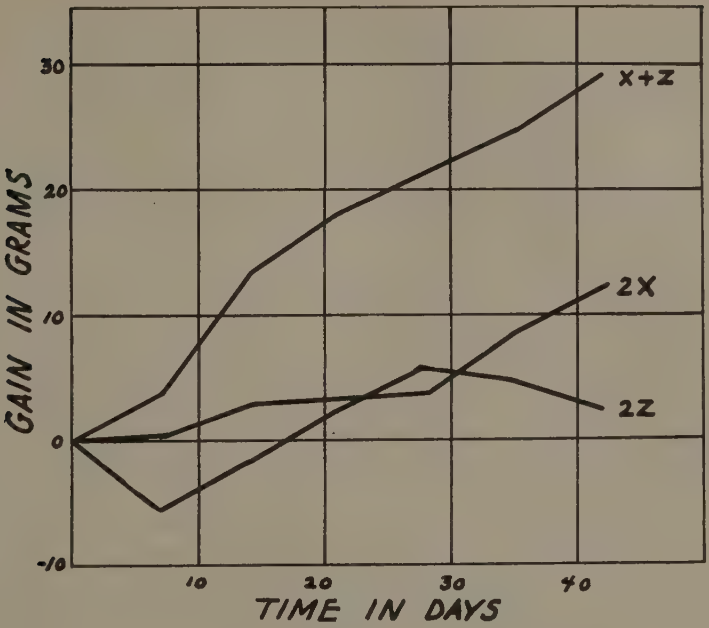

FIG. 2.

Gain curves of rats between their 14th and 20th week of life showing, in vitamin G tests, a supplementary effect of two products upon growth. X is an extract of skim milk powder made at room temperature with 80% alcohol; Z is the filtrate left after treating with Lloyd's reagent, an extract of skim milk powder made with boiling 95% alcohol. Each curve represents averages of 3 or 4 experiments.

an extract made from skim milk powder by means of boiling 95% alcohol; and (*c*) a mixture ($x + z$) of half of each of the quantities of products just described. One product was, apparently, a relatively good source of vitamin G, and a poor source of the "new" factor; the other, a relatively good source of the "new" factor and a poor source of vitamin G. The mixture promoted much better growth in these older rats than double the quantity of either product by itself.

The successful demonstration of supplementary effects on growth of these products was partially contingent on the use of experimental animals whose bodily store of the "new" factor was fairly low. The animals used in these experiments were reared by mothers receiving a diet consisting essentially of ⅙ whole milk powder and ⅚ ground whole wheat. But the attempt to use as vitamin G reagents, rats with this nutritional history, rather than those with a more optimal one, as is customary in most vitamin studies in this laboratory, also led to the discovery of a second type of evidence on the existence of "new" factors necessary to mammalian nutrition.

In the development of their method for the determination of vitamin G, Bourquin and Sherman[1] used as reagents, animals reared by mothers receiving a diet composed essentially of ⅓ whole milk powder and ⅔ ground whole wheat with or without the addition of raw lean beef. When the young with this nutritional history, depleted of their bodily reserves of vitamin G by a basal diet free from that vitamin, were given graduated portions of milk as vitamin G-containing food, they made a continuous and uniform growth response throughout an 8-week test period. No marked cessation of, or change in the rate of, growth was observed when the gains were averaged for large numbers of animals. But on using experimental animals with a less favorable nutritional history, employing otherwise the same experimental procedure, we found that only for the first few weeks of the test period did practically all of the animals respond in growth as did those of Bourquin, and only about one-third of more than 100 animals responded thus throughout an 8-week period. Instead of continuous and uniform growth, a flattening of the growth curve or a decline in weight was frequently observed after the 4th or 6th week of the experimental period. This result may undoubtedly be attributed to a depletion of the bodily store of some factor or factors other than those generally recognized as necessary to mammalian nutrition.

The main difference between the family diets on which the 2 groups of rats were reared consisted of the proportion of milk included. This and the fact that supplementary effects on growth in the course of vitamin G experiments may be observed with certain fractions of milk powder, when rats from family dietaries containing the less milk are used as reagents, indicate that milk is a fair source of the "new" factor. From the limited amount of evidence afforded by these experiments, it would appear that the "new" factor is less readily extracted than vitamin G from milk powder by alcohols 80% (by weight).

In the course of these and other experiments some evidence was also obtained of the existence of 2 other "new" factors, but the work of the author in these directions was discontinued because of transfer to a position at a distance from the laboratory. Evidence on the existence of accessory factors other than those generally recognized as necessary to mammalian nutrition has also been adduced by several other investigators. Palmer and Kennedy[2, 3, 4, 5], Salmon, Guerrant and Hays[6, 7], Hunt and his associates[8, 9], Chick and coworkers[10, 11, 12, 13], Coward and others[14, 15], Reader[16, 17, 18], Guha and Drummond[19, 20], Williams and Lewis[21], Halliday[22, 23], and others have found evidence of "new" factors or of factors "interfering" with the assay of the well-recognized factors. Only further research can determine whether the factors encountered in the work noted here are new in the sense of being different from factors already postulated by other investigators, or with which they are identical.

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6266

Dissociation of V. Cholerae by Bacteriophage.

W. K. CHEN. (Introduced by T. J. Kurotchkin.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College.

Bacteriophage is known to exercise a strong influence in enforcing microbic dissociation of certain bacterial cultures. Bacteriophage on *V. cholerae* produces phage resistant secondary cultures *in vitro* and certain coccoid mutants in convalescent cases (d'Herelle[1]). The variation of *V. cholerae* has also been reported by Baerthlein[2] Balteanu,[3] and others. The object of this study is to determine whether dissociation of *V. cholerae* by bacteriophage follows the same cycle proved for other motile bacteria, such as *B. paratyphosus A* by Arkwright[4] and hog-cholera bacillus by Li.[5] Morison's[6] combined phage of *V. cholerae* and *B. dysenteriae Shiga* was used for inducing dissociation. Two strains of *V. cholerae* susceptible to the phage were selected for study. One cc. of an 18-hour peptone water (pH 8.0) culture of each was transferred to a fresh peptone water medium to which half cc. of Morison's phage was introduced and incubated at 37°C. for one week. The process was repeated at weekly intervals, the cultures diluted, plated on semi-solid medium for examination of motility, and streaked on agar plates for the character of the surface colonies.

Variation in the appearance of the colonies appeared in the semisolid plates after 2 to 3 transfers followed gradually with more advanced changes as the transferring process went on, while the control tubes invariably gave one type of MS (motile smooth) colonies which grew deep, homogenous, similar to the smooth surface colonies on the agar plate (Fig. 1). Three distinct main types of variants arose from the phage resistant secondary cultures. After 2 to 4 weeks the first variant appeared from the secondary cultures as an irregularly shaped colony with clusters of small daughter colonies in the semi-solid medium (Fig. 2). The individual cells

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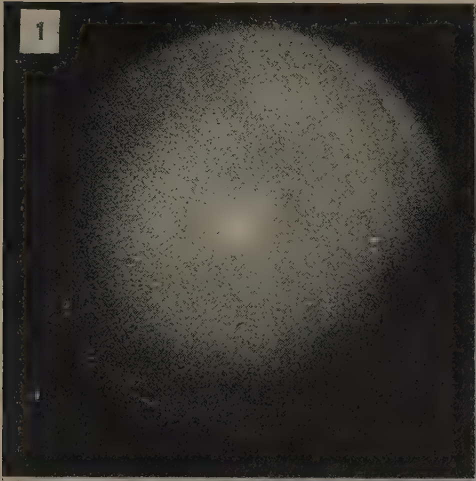
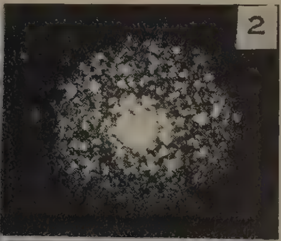




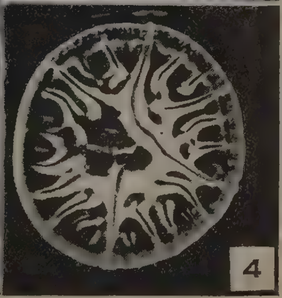


FIG. 1. Normal MS colony of *V. cholerae* 18 hours old, growing in semi-solid medium. ×15.

FIG. 2. MR colony 18 hours old in semi-solid medium, showing the cluster formation. ×15.

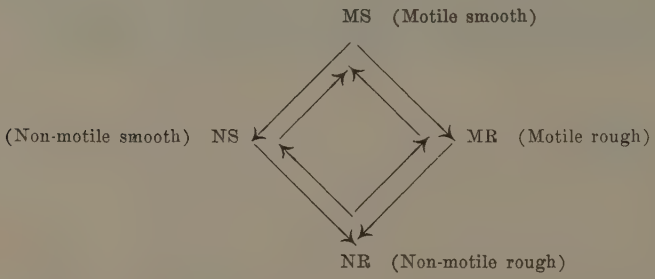
FIG. 3. NS colonies 48 hours old in semi-solid medium. NS and NR colonies appear alike under these conditions. ×15.

FIG. 4. NR colony 24 hours old on agar plate. MR and NR surface colonies appear alike under these conditions. ×15.

were motile but they formed typical rough, opaque surface colonies on agar plate and constituted the MR (motile rough) colonies. After 4 to 6 weeks the second variant appeared as very small compact colonies in the semi-solid medium, but formed typical surface smooth colonies on agar plate. They were non-motile and termed the NS (non-motile smooth) colonies (Fig. 3). After 6 to 10 weeks the third variant appeared as deep colonies similar to the NS type in semi-solid medium, but gave rough surface colonies similar to the MR type on agar plate (Fig. 4). It was the most stable variant and named as NR (non-motile rough) colony. Both rough variants gave granular growth in broth and were spontaneously agglutinated in saline, while both smooth forms grew homogenously in broth and formed stable suspensions in saline.

By daily transfers on agar slants the MR type showed a tendency to revert to MS type after 46 transfers; the other 2 types remained stable. But when the variants were repeatedly grown in peptone water and transfers were made twice a day for 30 days all the variants showed a tendency to reversion. Typical MS colonies appeared in the MR culture after 34 transfers in peptone water (twice a day) and also a few motile ones from the non-motile types after 60 transfers (NS to MS and NR to MR). Reversion of the variants was more evident by animal passage than by laboratory media. The MR culture showed some reversion to smooth after 6 animal passages, while after 10 passages some MS colonies appeared in the NS culture and both MR and NS forms from the NR culture.

This study seems to suggest the NR type is the direction towards which dissociation of *V. cholerae* leads and that the MR and NS forms are simply intermediate types. All the MS forms induced back from the variants by any process are as susceptible to the same phage action as the original culture. The dissociation induced by bacteriophage and the reversion of variant by animal passage as observed may be represented by the following diagram:



Organisms from all the 4 types were gram negative slender rods and culturally were typical. Virulence tests on hamsters (Chinese field mice) showed that, the variants were comparatively less virulent than the original MS culture, especially the NR type which was practically avirulent. Serologically there was very slight difference among the 4 forms except that the NR type gave an agglutination of a very low titer with the anti-sera of the 4 types.

6267

Modification of Van Slyke's Gas Apparatus for Determination of CO_2 in Muscle and Other Tissues

HSIEN WU AND EN-FU YANG.

From the Department of Biochemistry, Peiping Union Medical College.

For the determination of CO_2 in muscle and other tissues it is obviously necessary to modify the gas burette of Van Slyke and Neill[1] so that relatively large pieces of material can be introduced into the burette. Ferguson and Irving[2] have proposed to divide the burette into 2 separate parts just below the 2 cc. mark, the 2 parts being connected by means of a ground joint and held together with a pair of springs. In our laboratory several modifications have also been used. The one that seems most satisfactory is shown in Fig. 1.

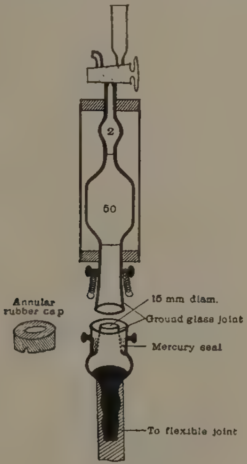


FIG. 1.

Modified gas burette for determination of CO_2 in muscle.

The new feature of the burette is the enlargement of the diameter of the lower stem of the burette and the introduction of a 15 cc. bulb between the burette and the flexible point. The short stem of the bulb is ground on the outside to fit the lower stem of the burette which is ground on the inside. The joint is surrounded by a cup for mercury seal and the cup is provided with an annular rubber cover to prevent the escape of mercury during shaking.

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For the determination of CO_2 about 0.5 gm. of muscle is taken from the animal and immediately put into a weighed, stoppered test tube containing a solution which stops respiration, hinders lactic acid formation and prevents loss of CO_2. The solution is made by mixing 1 cc. of 0.3 N CO_2-free NaOH and 3 cc. of 0.8% saline containing 65 mg. KCN and 1 gm. NaF per liter, and 2 drops of 0.1% phenol red solution. The tube is weighed again to obtain the weight of the muscle.

The material to be analyzed is introduced into the bulb, the burette is placed on it, stop-cock vaseline being used at the ground glass joint. The bulb and the burette are fastened by a pair of springs. With the cock of the burette open, mercury is admitted into the bulb pushing the material up into the burette and filling it completely with mercury. The tube in which the muscle has been weighed is rinsed with 2-m cc. of H_2O, m being the weight of muscle. The rinsing is introduced into the apparatus in the usual manner, followed by 2 drops of caprylic alcohol and 1 cc. of 1-N lactic acid. The total volume of solution plus that of muscle is 7 cc. The extraction is carried out in the usual manner.

With a 0.5 gm. sample of muscle, we find it necessary to extract for 30 minutes in the vacuum. During the extraction the reacting mixture was drawn once into the lower chamber to rinse it. The burette was allowed to stand for 15 minutes without shaking and then shaken for 15 minutes. Testing with a known solution of Na_2CO_3 we found no difference in the result whether the solution was introduced into the bulb or in the usual way. CO_2 was absorbed with 1 cc. gas-free 1-N NaOH solution. The calculation was done according to the method of Van Slyke and Neill, 1 gm. muscle being regarded as equivalent to 1 cc. of solution.

Our modification of the apparatus has 2 advantages over the form proposed by Ferguson and Irving. First, there is no danger of gas leaking into the chamber during extraction and second, the burette can be water-jacketed as in the usual form of the apparatus.

6268

Effect of Acidosis and Alkalosis on the Total Base, Chloride and CO_2 Contents of Muscle

EN-FU YANG AND HSIEN WU

From the Department of Biochemistry, Peiping Union Medical College.

It was reported[1] that the total base content of the abdominal muscle of rabbits receiving an injection of 25% NaCl in the pleural cavity was considerably higher than that of control animals receiving the same quantity of normal saline. The permeability of the muscle to the sodium ion having been thus demonstrated, the total base content of muscle might be expected to increase with alkalosis and to decrease with acidosis. The experiments reported in this paper are designed to test this point. Some further experiments with NaCl injection are included.

Rabbits were anesthetized with morphine hydrochloride (20 mg. per kg.). The abdominal wall was slit open, the ureters were tied, and the abdominal wall closed. Acidosis or alkalosis was produced by giving HCl, $NaHCO_3$ or Na_2CO_3 solution. In some experiments the solution was introduced into the stomach by means of a tube, in others it was injected into the femoral vein at a rate of 1 cc. per minute. One-half to 2 hours after the introduction of the solution, blood was drawn from the heart and mixed with ammonium oxalate under oil. Pieces of muscle were taken from the abdominal wall. A small portion of the blood which was collected separately under oil with potassium oxalate was used for the determination of pH by the method of Shock and Hastings[2]. The remainder of the blood was centrifuged under oil. Determinations of water, total base, Cl and CO_2 were made on the muscle and blood plasma. Water was determined by drying at 110° C. for 12 hours. Chloride was determined by the method of Wilson and Ball[3], total base by the method of Stadie and Ross[4]. CO_2 was determined by the manometric method of Van Slyke and Neill[5]. The special burette described in the preceding paper was used for the muscle. The results are summarized in Table 1 which includes some experiments with intra-

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TABLE 1

Total base, chloride, and carbon dioxide of plasma and muscle in acidosis and alkalosis (Millimols per kg.).

Route	No. experiments	Amt. and conc. of sol'n used per kg.	Time allowed for distribution, hr.	Plasma						Muscle			
				pH range	Average pH	H_2O	Base	Cl	CO_2	H_2O	Base	Cl	CO_2
Normal	6			7.36-7.48	7.40	94.0±0.3	162.5±2.7	111.2±2.0	22.5±1.3	74.9±0.1	132.0±1.5	16.7±0.7	12.2±0.3
Oral													
$NaHCO_3$	9	50 cc 10%	2	7.42-7.62	7.50	93.8±0.3	187.5±5.0	93.6±2.5	39.0±2.0	72.7±0.4	145.7±2.6	13.7±0.5	14.8±0.5
HCl	7	50 cc 0.3 N	2	6.80-7.22	7.00	93.7±0.2	152.9±1.9	113.8±2.7	10.8±2.0	74.6±0.7	129.4±1.5	17.7±0.7	10.5±0.3
Intravenous													
Na_2CO_3	4	10 cc 1-N	½	7.50-7.67	7.59	94.4±0.2	172.4±3.8	98.5±1.2	37.0±1.5	71.7±0.5	129.1±1.8	15.6±0.6	14.0±0.5
*HCl	4	5 cc 1-N	½	7.22-6.77	7.06	93.8±0.5	163.3±0.5	115.7±3.7	15.5±2.6	74.4±0.5	121.0±2.7	18.4±1.1	9.7±7.0
0.8% NaCl	4	10 cc 0.8%	½	7.28-7.40	7.36	94.9±0.5	159.6±0.6	111.8±2.1	24.7±2.1	71.3±1.0	119.2±2.2	16.3±0.9	11.1±0.4
20% NaCl	3	10 cc 20%	½	7.02-7.22	7.15	94.0±0.3	215.6±1.4	167.1±2.1	12.3±1.3	69.2±1.0	145.1±1.0	30.3±0.4	8.9±0.3
Subcutaneous													
25% NaCl	9	10 cc 25%	2			94.6±0.2	190.6±9.5	158.0±9.0		72.4±0.7	153.8±3.8	27.4±3.2	
0.8% NaCl	5	10 cc 0.8%	2			94.0±0.3	165.1±3.8	105.7±2.5		74.4±0.4	131.9±2.0	16.5±1.4	

*Ureters not tied.

venous injection of NaCl. These experiments confirm our previous findings.

It will be noted that in alkalosis there is an increase in the total base content of the muscle. The increase of base in muscle as well as in plasma is more marked when the base was introduced orally than intravenously. This might be due to the fact that larger doses were used for oral administration. There is also a decrease of Cl content in the muscle in alkalosis produced by oral administration of alkali, but intravenous injection of alkali has no significant effect on muscle Cl. No significant change in muscle base and Cl was observed in acidosis. The changes in CO_2 content of the muscle are just what we might expect. The water content of the muscle is not significantly changed by our experimental procedure.

The alkalosis produced in our animals was accompanied by an increase of base and decrease of chloride in the plasma, but the acidosis was not accompanied by the reverse effect. It appears therefore that the base and chloride contents of muscle vary with the base and chloride concentrations of the plasma, but they are not significantly influenced by pH changes as such.

6269

Isolation of Treponema pallidum from Juxta-articular Nodules

CH'UAN-K'UEI HU AND CHESTER N. FRAZIER.

From the Peiping Union Medical College.

The syphilitic nature of the juxta-articular nodules occasionally seen in syphilitic patients is generally accepted principally because of the prompt response of the lesions to arsphenamine therapy. Since the nodules are usually encountered in patients in the later stages of the infection, the failure to demonstrate organisms by the dark-field or in stained tissue sections is presumably due to their scarcity. Animal inoculation offers a more hopeful means of success and although the method has apparently not been widely used, one successful result has been reported[1]. This method was employed in 2 of the 5 cases of juxta-articular nodes in syphilitic patients seen in the Peiping Union Medical College[2]. One attempt was successful;

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[1] Jessner, M., and Rossiansky, N., *Arch. f. Derm. u. Syph.*, 1930, **160**, 224.

[2] Hu, C. K., and Frazier, C. N. *Chinese Med. J.*, in press.

the failure in the second case may have been due to the fact that arsphenamine had been administered 3 days before admission.

The patient gave a history of venereal exposure and a genital chancre 12 years before admission; the blood Wassermann and Kahn reactions were positive. There were several small firm, non-tender nodules in the subcutaneous tissue below both elbows and over the great trochanters. One nodule (1.0 x 1.0 cm.), removed under local anesthesia, was finely emulsified with sterile normal saline. All the suspension was used for inoculation, 1.0 cc. being injected into the right testicle of 2 normal rabbits. In both animals, an orchitis developed 10 weeks after inoculation and the blood Wassermann reaction became positive. In one rabbit, *Treponema pallidum* were demonstrated in the material aspirated from the testicular lesion; a metastalic lesion of the uninoculated testicle developed 3 weeks after the primary orchitis was detected. Spirochetes were not seen in the material aspirated from the second rabbit which died from an intercurrent infection.

The result of this work supports the opinion that juxta-articular nodules in syphilitic patients are syphilitic lesions. Although a blood stream infection was not excluded in the present instance, its possibility in a disease of 12 years' standing is unlikely.

New York Meeting.

6270

Simplified Colorimetric Determination of Blood Urea Clearance.

DONALD D. VAN SLYKE AND C. L. COPE.

From the Hospital of the Rockefeller Institute for Medical Research, New York.

The urea contents of urine and blood are compared in a colorimeter in such a manner that a single reading gives directly the percentage of average normal renal function in terms of the blood urea clearance.[1] The urine is first diluted to such an extent that if the clearance, either standard or maximum, is the average for a normal subject, the urea concentrations in blood and diluted urine will be equal. The urea in both blood and urine is converted into ammonia with urease; proteins and other interfering substances are removed, and the ammonia contents of the 2 filtrates are compared colorimetrically.

No standard solutions are required, because the blood is compared directly with the urine.

The number of times the urine must be diluted for comparison with the blood is found by reference to the curve of Fig. 1, computed as follows: The average *maximum* clearance (for urine volumes over 2 cc. per minute) is 75. The maximum clearance is calculated as $C_m = \frac{U\ V}{B}$ where V is reckoned in cc. of urine per minute, B is blood urea concentration, and U is urine urea concentration. When C_m has the average normal value of 75, the ratio U/B is calculated therefore as $U/B = 75/V$. When V is expressed in cc. of urine per hour, the calculation changes to $U/B = 4500/V$. The ratio U/B is the number of times a portion of the urine must be diluted to bring its urea concentration down to that of the blood, when the clearance is average normal. The U/B values thus calculated are expressed by the higher part of the curve of Fig. 1.

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[1] For a discussion of the significance of the blood urea clearance, see Møller, McIntosh and Van Slyke, *J. Clin. Inv.*, 1928, **6**, 427; or Peters and Van Slyke, *Quant. Clin. Chem.*, 1931, **1**, 345.

If the urine volume is below 2 cc. per minute, the formula which holds is that of the standard clearance, $C_s = \frac{U\sqrt{V}}{B}$, and the average normal value of C_s is 54. The number of times dilution required for urines excreted at rates less than 2 cc. per minute is therefore calculated as $U/B = 54/\sqrt{V}$ when V is expressed in cc. of urine per minute, or as $U/B = 54 \times \sqrt{60}/\sqrt{V} = 418\sqrt{V}$, when V *is* expressed in cc. of urine per hour The dilution ratios thus calculated are indicated by the lower part of the curve of Fig. 1. Plotting the curve logarithmically gives it a rectilinear form, with a break at the augmentation limit where the C_s formula is replaced by the C_m formula.

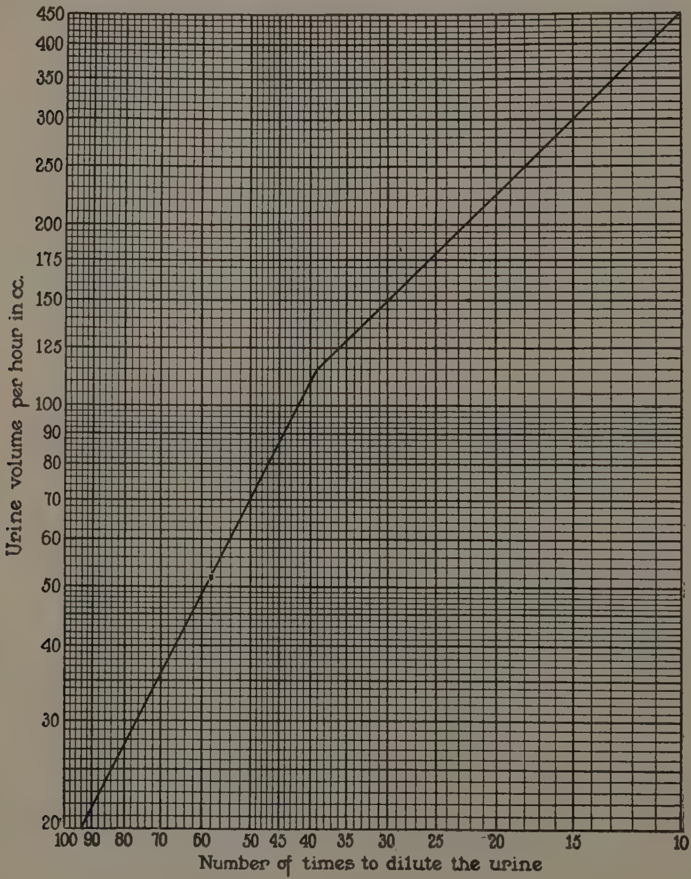

FIG. 1.

Reagents.

Permutit;[2] *Urease.* 10% solution of Squibb's urease;[3] *phosphate buffer*, 5 gm. of KH_2PO_4, and either 5 gm. of $Na_2HPO_4.12H_2O$ or 2 gm. of anhydrous Na_2HPO_4, per 100 cc. *Somogyi's acid zinc sulfate solution* for precipitating proteins.[4] 12.5 gm. of $ZnSO_4.7H_2O$ and 125 cc. of 0.25 *N* sulfuric acid diluted to 1 liter. 0.75 *N sodium hydroxide. Nessler's solution.*[5] *Sodium citrate,* pulverized.

Conditions for collecting blood and urine. Urine is collected during 2 successive periods of one hour each, and the volume in each hour is accurately measured. Blood is drawn at about the middle of the 2-hour period. The blood is collected with citrate, if possible, rather than oxalate as anticoagulant, because oxalate tends to produce turbidity in the blood filtrate when the latter is nesslerized.

Treatment of Urine.

Removal of preformed ammonia. Of the urine collected during each hour, place 10 cc. in a small Erlenmeyer flask with 2 gm. of permutit and rotate for 5 minutes to absorb ammonia. Filter through a dry filter paper.

Treatment with urease. Place in a 100 cc. cylinder a volume of permutit-treated urine, 1, 2, or more cc., such that when later diluted according to Fig. 1, the resultant volume will be between 50 and 100 cc. To the undiluted urine in the cylinder add 5 cc. of the phosphate buffer solution and 1 cc. of the 10% urease. Let stand 10 minutes, preferably not much longer, in order not to give ammonia opportunity to volatize.

Dilution and removal of protein and other interfering substances. After the urease has acted 10 minutes 3 cc. of the zinc sulfate solution are added. It reacts with the phosphate buffer to produce a precipitate which clears the urine. The mixture is then diluted up to the volume calculated from Fig. 1. After standing a few minutes, the solution is passed through a dry filter. A slight cloudiness may be present in the filtrate, but the turbidity disappears when the filtrate is nesslerized. A portion of the filtrate, 2 or 5 cc., is pipetted into a volumetric flask and diluted 10 times, in order to parallel the 10-fold dilution which the blood undergoes.

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[2] Folin, O., and Bell, R. D., *J. Biol. Chem.*, 1917, **29**, 329.

[3] Prepared from jack beans by the method of Van Slyke and Cullen, *J. Biol. Chem.*, 1914, **19**, 211.

[4] Somogyi, M., *J. Biol. Chem.*, 1930, **86**, 655.

[5] Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **46**, 2066.

If the subject is a child, or deviates greatly from usual adult size, a correction for body size is introduced by multiplying the observed volume of the hour's urine by the proper factor from Fig. 1 of McIntosh, Møller, and Van Slyke,[6] and using the urine volume thus multiplied as the ordinate for Fig. 1 of this paper. For example, if the urine volume per hour is 80 cc., Fig. 1 indicates that the urine should be diluted 47 times. This holds for any adult within usual size limits. If, however, the subject is a child of 1.40 meters height, the 80 cc. observed hour's volume is multiplied by the factor 1.49 from McIntosh, Møller, and Van Slyke.[6] The corrected volume, 119 cc., is then interpolated on the curve of Fig. 1, and indicates that the urine should be diluted only 37.5 times.

Treatment of Blood.

Digestion with urease. The volume of blood sample required depends upon the volume of solution necessary for a reading in the colorimeter used. If 5 cc. of solution suffice for the colorimeter, one cc. of blood will do, unless an unexpectedly low clearance value necessitates the use of a fresh portion of filtrate for supplementary dilution. In general, a 2 cc. sample is adequate. The sample is mixed with 0.1 its volume of the 10% solution of urease, and let stand 10 or 15 minutes.

Removal of blood proteins. For each cc. of blood sample, 7.9 cc. of Somogyi's acid zinc sulfate solution are added, then 1 cc. of 0.75 N sodium hydroxide. After standing for some minutes the fluid is passed through a dry filter.

Nesslerization and Comparison of Blood and Urine Filtrates.

Equal volumes of the blood filtrate, 5, 10, or 20 cc., as required by the colorimeter, and of the 10-fold diluted urine filtrate are pipetted into dry vessels, and a trace of sodium citrate powder is added from the point of a knife to each and allowed to dissolve. The citrate retards development of turbidity after nesslerization To each measured portion of filtrate one-tenth its volume of Nessler's solution is added, and the solutions are at once compared in a colorimeter. The nesslerization may be done in the colorimeter cups if they are dried before the filtrates are measured in. The reading must be taken quickly after nesslerization, or turbidity may develop in the blood filtrate, making the blood urea content appear too high, and the clearance too low.

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[6] McIntosh, J. F., Møller, E., and Van Slyke, D. D., *J. Clin. Invest.*, 1928, **6**, 467.

The use of the urease method for comparing contents of urea in the blood filtrate and diluted urine is not obligatory. Any other procedure which will give direct comparison with sufficient accuracy may be substituted.

Calculation. If the colors of the 2 filtrates are such that the higher scale reading does not exceed twice the lower, the clearance is calculated directly from the colorimeter scale readings. *% of average normal clearance* $= \frac{100\,(B)}{(U)}$. (B) represents the reading of the blood filtrate, and (U) the reading of the urine filtrate. (The readings are inversely proportional to the concentrations, hence the inversion of the usual concentration formula.)

If the clearance is less than half average normal, the blood filtrate when nesslerized will be more than twice as deep in color as the urine filtrate. In such a case the preliminary $(B)/(U)$ reading is made in the above manner. Then a fresh portion of the blood filtrate is diluted 2, 3, 5, or 10 times, as indicated by the preliminary reading in order to bring the $(B)/(U)$ ratio into the neighborhood of unity. A portion of the diluted blood filtrate is then nesslerized and compared with a fresh portion of the urine filtrate simultaneously nesslerized. The calculation then becomes: *% of average normal clearance* $= \frac{100\,(B)}{D\,(U)}$. D represents the number of times the blood filtrate has been diluted before the final nesslerization, in cases where a low clearance necessitates such dilution. If the subject is known to have a low clearance, the time required for the preliminary reading may be saved by diluting the blood filtrate to the extent probably necessary before the first reading is made.

TABLE I.

Subject No.	% of Average Normal Clearance		Deviation of Colorimetric from Gasometric in Percentage of Gasometric Clearance
	Colorimetric	Gasometric	
1	153.9	145.7	+5
2	141.0	131.8	+7
3	84.2	80.0	+5
4	56.9	59.3	—4
5	49.6	48.0	+3
6	46.2	49.2	—7
7	40.2	37.6	+6.5
8	40.1	44.7	—11
9	38.4	39.9	—4
10	38.1	35.8	+7
11	37.3	38.7	—4
12	36.5	33.7	+8
13	34.8	32.1	+8
14	32.1	35.1	—6
15	28.0	28.6	—2

Results. Urea clearances in normal subjects and nephritic patients have been determined by the above method, and compared with clearances calculated from urea determinations made on the same blood and urine by the exact gasometric urease method.[7] Table I gives a representative series of results. The first 3 determinations were on a hypertension patient with normal renal function; the other determinations were on nephritics with diminished renal function.

6271

Is Erythropoietic Action of Copper Dependent upon Presence of Adequate Supply of Iron in Diet?*

HERMANN B. STEIN AND ROBERT C. LEWIS.

From the Department of Biochemistry, University of Colorado School of Medicine, Denver.

In studying the effect of evaporated milk upon the development of nutritional anemia in the albino rat, it was observed that the erythrocytes were maintained at a normal level over a period of 8 weeks in spite of the fact that the hemoglobin fell slowly. This was in sharp contrast to the findings on rats which were fed raw milk, where both the erythrocyte count and the hemoglobin showed a marked decrease in 7½ weeks.

In the majority of evaporated milk plants the milk comes in close contact with copper during the manufacturing process. Since according to Rice and Miscall[1] copper is dissolved by milk under such conditions, an investigation has been undertaken to determine if this metal is the causative factor in the maintenance of the red cell count when evaporated milk is fed.

Albino rats, 21 days of age, were fed raw milk supplemented by 0.025 mg. of copper (as $CuSO_4$) daily over a period of 8 weeks. Erythrocyte counts and hemoglobin determinations were made at the beginning of the experiment and at intervals of 2 weeks thereafter. The experimental data obtained, with those from rats on raw milk alone and on evaporated milk without supplement, are given in Table I.

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[7] Van Slyke, D. D., *J. Biol. Chem.*, 1927, **73**, 695.

* This work was aided by a grant from the Evaporated Milk Association.

[1] Rice, F. E., and Miscall, J., *J. Dairy Sci.*, 1923, **4**, 261.

TABLE I.

Diet	No. of Rats	Av. Weight (gm.) Init.	Av. Weight (gm.) Final†	Av. Hemoglobin (gm. per 100 cc.) Init.	Av. Hemoglobin (gm. per 100 cc.) Final†	Av. Erythrocytes (per cu. mm.) Init.	Av. Erythrocytes (per cu. mm.) Final†
Raw Milk	5	33.0	59.0	14.01	2.18	6,770,000	2,750,000
Evaporated Milk	5	35.3	87.9	12.07	5.81	6,560,000	7,220,000
Raw Milk + Cu	6	35.8	66.6	10.39	4.13	5,591,000	4,786,000

† At end of 8 weeks except for animals on raw milk alone (7 weeks, 3 days).

One may conclude that the maintenance of a normal erythrocyte count for 8 weeks when evaporated milk is fed as the sole article of diet is due in part at least to the copper content of the evaporated milk, as a similar though less marked effect is obtained when raw milk is supplemented with copper.

The recent report of Schultze[2] that, even when the diet is poor in iron, copper fed to young anemic rats has an erythropoietic action without stimulating hemoglobin regeneration is supported by our experimental findings.

6272

Determination of Colloid Osmotic Pressure of Blood Serum.

MARVEL-DARE FELLOWS.* (Introduced by Wm. S. McCann.)

From the Department of Medicine, University of Rochester School of Medicine and Dentistry, and the Medical Clinic of the Strong Memorial Hospital, Rochester, N. Y.

Results of previous workers from Starling[1] to Krogh and Nakazawa[2] have shown the colloid osmotic pressure of the blood serum to vary between 265-420 mm. of water pressure, with average values of approximately 310-360 mm.

Osmotic pressures reported here have been obtained with a greatly modified apparatus using aseptic technique, an accurately controlled constant temperature water bath at 30°C., and modified Ringers as a dialyzing agent. The figure shows osmometer tube (C) with (Dupont No. 300 plain transparent cellophane) membrane attached, which is filled with blood serum; (D) is capillary tube attachment for noting blood serum meniscus; (E) is stopcock

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[2] Schultze, K. W., *Klin. Wchnschr.*, 1932, **11**, 497.

* Aided by a grant from the Fluid Research Fund of the Rockefeller Foundation.

[1] Starling, E. H., *J. Physiol.*, 1896, **19**, 312; 1899, **24**, 317.

[2] Krogh, A., and Nakazawa, F., *Biochem. Z.*, 1927, **188**, 241.

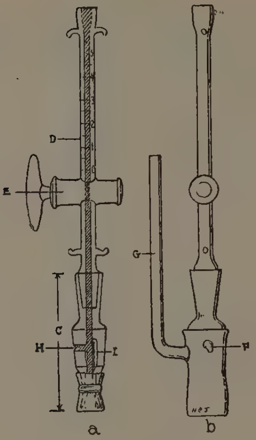

FIG. 1.

to prevent rise of serum in capillary during development of pressure. (A) is fitted into (B) which shows saline tube (F) with side arm (G) for filling or for removing bubbles from serum tube through orifice (H) or side arm can be turned to groove (I) allowing air to enter outer system, thus preventing the production of a vacuum. The 3 parts are fastened together by means of springs over small glass hooks. The osmometer is immersed in a constant temperature bath up to the stopcock and attached to a manometer.

It has been inferred that a low serum protein value means a lowered serum osmotic pressure. Accordingly, a short series of readings was done on dog serum obtained from dogs which were undergoing plasma-pheresis[3] and whose serum protein values were consequently much lower than those of normal dogs, being from 3.65 to 5.27 gm. per 100 cc. of serum as compared with 6.35 to 6.60 gm. per 100 cc. of serum for the normal animals. The serum osmotic pressures (measured by Krogh's method[4]), when compared with similar determinations on normal dogs, were also found to be definitely lower, 93 to 225 mm. of water pressure with normal values showing 292 to 334 mm. of water pressure; however, it is now felt that the determinations may have been somewhat inaccurate because of the method used. At the time the above observations were made there had been no attempt either to observe sterile precautions in handling serum or to maintain a uniform temperature throughout the experiment. Elimination of these variables seems highly desirable.

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[3] Lepore, M. J., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 318.

[4] Krogh, A., The Anatomy and Physiology of Capillaries, Yale U. Press, 1922.

Determinations of osmotic pressures of 11 apparently normal medical students give values ranging from 321 to 380 mm. of water. The serum albumin and serum globulin were determined by analysis using the method of Wu and Ling[5] and give values of 6.32 to 6.81 gm. of protein per 100 cc. of serum. In calculating osmotic pressures from these protein values the data of Govaerts were used (75.4 mm. of water pressure per gm. of albumin and 19.5 mm. of water pressure per gm. of globulin) and the osmotic pressures were found to be 361 to 394 mm. of water, values which are consistently higher than those obtained by direct measurement.

Our results indicate a fair degree of accuracy and it is hoped that with the accumulation of more extensive data a better correlation between observed and calculated osmotic pressures may be obtained.

Thanks are due to Prof. W. R. Bloor of the Department of Biochemistry and to Dr. Samuel H. Bassett of the Department of Medicine for much helpful advice, and to Miss Clara M. Husted for technical assistance.

6273

A Note on the Minimal Fatal Dose of Nicotin for Unanesthetized Dogs.

F. E. FRANKE AND J. EARL THOMAS.

From the Department of Physiology, St. Louis University School of Medicine, and Jefferson Medical College.

The following observations were made as part of a study of the cause of death in nicotin poisoning in dogs. Sollmann[1] mentions that 2 drops of nicotin placed on the gums of a dog may rapidly cause death. Blake[2] injected 2 drops of nicotin into the jugular vein of a dog, death occurring about one minute and a half after the administration of the poison.

At the time we carried out our experiments we were not aware of any reports in the literature dealing with the minimal fatal dose of nicotin for dogs. von Anrep[3] reports that 1.5 drops of nicotin is a dangerous dose and 3.5 drops is fatal in a few minutes, for dogs weighing from 10 to 17 kg. Expressed in mg. of nicotin per kg. of dog's weight, the dangerous dose would be approximately 5.7 to 9.75 mg. per kg. and a dose of 13.4 to 22.75 mg. per kg. would be fatal in a few minutes. Kobert[4] places the minimal fatal dose at 0.5 to 2 drops of nicotin.

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[5] Wu and Ling, *Chinese J. Physiol.*, 1927, **1**, 161.

[1] Sollmann, Manual of Pharmacology, 1922, Saunders, Philadelphia.

[2] Blake, *Edinb. Med. and Surg. J.*, 1840, **53**, 35.

[3] von Anrep, *Arch. f. Physiol.*, Leipz, 1879, Suppl. Bd., 167-191.

Nicotin was administered to 48 dogs. In a majority of the experiments no drug other than nicotin was given. In a few cases a small amount of novocain was used to produce local anesthesia for the exposure of a vein or artery. In some the blood pressure was recorded, but without the use of any anticoagulant. Neither the minor operative procedures nor the local anesthetic had any observable influence on the results.

Three methods of administering the drug were used:

1. By mouth. Nicotin was dropped on the tongue or between the lips and gums in the form of the undiluted alkaloid.
2. By injection into the circulating blood. The alkaloid in 10% solution in Locke's fluid was injected into a vein (with or without surgical exposure) or directly into the cavity of the left ventricle.
3. Subcutaneously or intramuscularly, in 10% solution of the hydrochloride.

The results are summarized in Table I. The smallest dose of nicotin which proved fatal in a high percentage of dogs was, when given by mouth, 10 mg. per kg., and when injected into the circulation, 3 mg. per kg. Doses larger than 12 mg. per kg. by mouth and 5 mg. per kg. injected into the circulating blood were uniformly fatal. The fatal dose when given subcutaneously was not ascertained but 23 mg. per kg. failed to cause death. In one dog, 7.7 mg. per kg. intramuscularly proved fatal. The nicotin for intramuscular and subcutaneous administration was measured as the alkaloid but administered as the hydrochloride.

There was considerable individual variation in susceptibility. Two dogs recovered after receiving 5 mg. per kg. into the circulation, and one animal died after receiving only 2 mg. per kg. It happened also that all of the animals in this group that received 3 or 4 mg. per kg. died.

The last column of the table gives the time elapsing after giving the nicotin until the palpable pulse and respiration ceased. Most of the animals died in 4 minutes or less. Those given the drug intravenously died in about 2 minutes. It is remarkable that in these animals there is no appreciable difference in the time till death corresponding to the

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[4] Kobert, R., *Lehrbuch der Intoxikationem*, 1906, Bd. 2.

TABLE I.

Administration	Dose	No. of experiments	No. fatal	No. non-fatal	% fatal	Average time till death
	(mg. per kilo)					minutes
By mouth	20	2	2	0	100	2.5 (1 exp.)
” ”	12	1	1	0	100	3.5
” ”	9.2 to 10.3	14	8	6	57.1	3.77
” ”	4.6 to 5.0	2	0	2	0	
Intracardiac	5.7 to 6.0	3	3	0	100	2.5
”	5	1	0	1	0	
Intravenous	10	5	5	0	100	1.93 (4 exps.)
”	5	7	6	1	85	1.96 (5 exps.)
”	4	1	1	0	100	2.00
”	3	3	3	0	100	1.88
”	2	4	1	3	25	2.3
Subcutaneous	23	1	0	1	0	
”	15	1	0	1	0	
”	9	1	0	1	0	
”	8.8	1	0	1	0	
Intramuscular	7.7	1	1	0	100	4.0

difference in dosage. Ten, 5, and 3 mg. per kg. caused death in an average time of 1.93, 1.96, and 1.88 minutes, respectively.

A study of the minimal fatal dose of nicotin when given intravenously to etherized dogs was previously reported.[5] Comparison of those results with experiments reported here indicates that small doses of nicotin (2 to 4 mg. per kg.) are more likely to prove fatal in normal than in anesthetized animals. For example, 4 animals out of 5 in the etherized series recovered following administration of 3 mg. per kg., whereas that dose was uniformly fatal (3 animals) in this series, and one animal out of 4 that received but 2 mg. per kg. died. Furthermore, the unetherized animals died much more quickly than the etherized animals. The average time till death after giving the drug in 12 etherized animals was 7.8 minutes. In the 24 animals of the present (unetherized) series that received the drug in the same way (into the circulating blood), the average time until death was 2 minutes.

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[5] Thomas, J. Earl, and Franke, F. E., *J. Pharmacol. and Exp. Therap.*, 1928, **34**, 111.

6274

Synergism of Morphine and the Mydriatic Alkaloids in the Albino Rat.

THEODORE KOPPANYI.

From the Department of Pharmacology and Materia Medica, Georgetown University School of Medicine, Washington, D. C.

The subcutaneous or intraperitoneal injection of 10 to 15 mg. of morphine sulphate per 100 gm. of body weight, causes under normal circumstances, no perceptible change in the size of the pupil of the albino rat. Such doses of morphine produce a state of catalepsy and stiffness of the tail in the experimental animal and abolish the corneal reflex.

To such morphinized animals several mydriatics were administered by different routes. At first, only sympathetic stimulants were used. Normal-control and morphinized rats were each given intermuscular injections of cocaine hydrochloride 10 mg. per 100 gm. of body weight. Solutions of cocaine (1:50), of adrenalin hydrochloride (1:1000), and of ephedrine sulphate (1:100) were instilled in the conjunctival sac. (In every series of experiments 12 rats were used.) These installations, or injections, produce no, or only slight, dilatation of the pupil in the normal untreated rats, but increase the size of the pupil from ½ to 4 mm. or more in the morphinized animals.

In another series of experiments, one drop of a 0.002% solution of atropine sulphate was instilled into the conjunctival sac. This installation produced no appreciable change in the normal pupil, but a 60% dilatation in the morphine-treated rats.

Since it is known that morphine causes a secretion of adrenalin from the adrenal gland, it was supposed that this exaggeration of the action of mydriatics by morphine is due to the adrenalin liberated into the blood stream and thus represents a case of additive effects. However, complete adrenalectomies carried out in 25 rats do not support this contention. Adrenalectomized rats under the influence of morphine are just about as sensitive to mydriatics as unoperated controls.

The depression of rats by chloral hydrate or paraldehyde does not enhance the mydriatic effects of adrenalin, cocaine, ephedrine or atropine.

We are forced to conclude that morphine potentiates mydriatic actions especially of sympathetic nature in the albino rat. The potentiation of other sympathomimetic drug effects by morphine is being investigated.

6275

Studies on the Emetic and Anti-Emetic Actions of Ergotamine.

THEODORE KOPPANYI AND EVERETTE I. EVANS.

From the Department of Pharmacology and Materia Medica, Georgetown University School of Medicine, Washington, D. C.

Eggleston and Hatcher[1] showed that intravenous doses of 60.0 mg. of fluid extract of ergot (in terms of the dry drug) produced prompt emesis in the dog, whereas oral administration of even massive doses failed. Eviscerated dogs also showed emesis following intravenous doses. The authors concluded that ergot has a central emetic action. Hatcher and Weiss[2] apparently rejected this supposition, stating "that ergotoxin has no perceptible effect directly on the vomiting center, and that any inhibition by it of the emetic action of substances used in these experiments, must be due to its causing depression of some peripheral structure."

These authors found that massive doses of ergotoxine prevented the emetic action of apomorphine, aconitin, digitalis, and pilocarpine by systemic administration, but not of large oral doses of mercuric chloride and tartar emetic. They concluded that the anti-emetic activity of ergotoxine is due to its depression of afferent sympathetic nerve ends.

Koppanyi[3] found a close parallelism to Hatcher and Weiss' results. His dogs, whose medullary vomiting centre was removed by ablation or depressed by local application of morphine, also failed to vomit following systemic administration of apomorphine, pilocarpine, digitalis, but vomited upon oral administration of tartar emetic, copper and zinc sulphate, and mercuric chloride.

The following experiments were performed to correlate the above findings. Doses are given in mg. × kilogram body weight. In dogs the intravenous administration of small doses (0.02-0.2 mg.) of ergotamine tartrate elicit repeated retching and vomiting, but large doses (0.5 mg. or higher) fail to produce retching or vomiting. In cats the minimum emetic doses are somewhat lower than in dogs (0.01-0.02 mg.) and doses of 0.3 mg. or higher fail to produce emesis. Dr. F. F. Yonkman of Boston University also has noted the emetic response to small intravenous doses in dogs. (Oral communication.)

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[1] Eggleston, C., and Hatcher, R. A., *J. Pharm. and Exp. Therap.*, 1915, **7**, 225.

[2] Hatcher, R. A., and Weiss, S., *J. Pharm. and Exp. Therap.*, 1923, **22**, 139

[3] Koppanyi, T., *J. Lab. and Clin. Med.*, 1930, **16**, 225.

In 2 dogs we failed to produce vomiting by application of ergotamine tartrate (0.03 mg.) on the Thumas centre. In 2 experiments we found that the minimum emetic dose is lower when injected into the externalized common carotid artery than when it is given intravenously. Application of larger doses (0.2 mg.) on the centre suppressed the usual vomiting responses following optimum intravenous doses of ergotamine tartrate and also of apomorphine. However, these dogs vomited following oral administration of copper sulphate.

Similarly, the subcutaneous injection of 20 mg. morphine sulphate inhibited ergotamine and apomorphine vomiting, but did not suppress copper sulphate emesis.

In each of 3 dogs we performed a bilateral vago-sympathectomy and transection of the spinal cord at the level of the second thoracic vertebra. Allowing sufficient time for recovery, each of them received an intravenous dose of 0.05 mg. ergotamine tartrate. In every instance the dogs vomited in 3½, 6, and 7 minutes after administration and several times after. Intravenous doses of 0.5 mg. pilocarpine hydrochloride also produced repeated retchings and vomitings.

These results require little interpretation. We are convinced that ergotamine and other systemically administered emetics act directly on the vomiting centre and that the lowering of the threshold of the vomiting centre to normal emetic impulses has little, if any, rôle in the production of "central emesis". Moreover, we feel the inhibition of vomiting by ergotoxine or ergotamine is due to depression of Thumas' centre, but not to paralysis of peripheral afferent structures.

The possible therapeutic applications of these data and a standardization of *Fluidextractum Ergotae* by the minimum effective emetic doses are being investigated.

6276

Studies on the Evacuant Action of Ergotamine.

EVERETTE I. EVANS AND THEODORE KOPPANYI.

From the Department of Pharmacology and Materia Medica, Georgetown University School of Medicine, Washington, D. C.

The studies of Hatcher and Weiss[1] and of Koppanyi[2] suggested the presence of a medullary defecation center, or at any rate, the existence of a non-peripheral control of defecation.

Investigating the gastro-intestinal actions of graded doses of ergotamine tartrate in intact, unanesthetized dogs and cats, we observed certain facts which further support the assumption of a central control of defecation.

Dogs and cats strain and defecate rather promptly following small or moderate intravenous doses of ergotamine tartrate. Doses are given in mgm. × kilogram body weight. In the dog doses of 0.02-0.2 mg. produced prompt and repeated straining and defecation, whereas doses of 0.5 mg. or above were effective only after a considerable delay. (10 min.) In cats, doses of 0.01 and 0.015 mg. also produced straining and defecation whereas doses of 0.1 mg. or higher either produced no defecation or a very delayed response (9-68 min.). Thus in cats the optimum effective doses are somewhat smaller than in dogs. It is also to be noted that there is a close correlation between the evacuation and emetic response produced by ergotamine tartrate. In practically every case where vomiting was produced, defecation also took place and when vomiting did not take place the animals either failed to defecate or evacuation ensued after considerable delay (peripheral effects?).

In 2 dogs we failed to produce defecation by application of ergotamine tartrate (0.03 mg.) on the floor of the fourth ventricle; but the application of larger doses (0.2 mg.) upon the floor inhibited the usual evacuation following optimum intravenous doses. Similarly, the subcutaneous injection of 20 mg. of morphine sulphate eliciting the usual immediate evacuant response, inhibited the evacuant effects of subsequent intravenous doses of 0.1 mg. of ergotamine tartrate.

In each of 3 dogs we performed a bilateral vago-sympathectomy and a high thoracic transection of the spinal cord at the level of the second thoracic vertebra. Allowing ample time for the animals to recover from the effects of anesthesia, each of them received an intravenous injection of 0.05 mg. of ergotamine tartrate. In every instance these injections failed to elicit defecation. However, upon intravenous injection of 0.5 mg. of pilocarpine hydrochloride defecation followed 3, 5, and 9 minutes after administration.

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[1] Hatcher, R. A., and Weiss, S., *J. Pharmacol. and Exp. Therap.*, 1923, **22**, 139.

[2] Koppanyi, T., *J. Lab. and Clin. Med.*, 1930, **16**, 225.

Not wishing to deny the probability of peripheral purging effects of ergotamine and other ergot principles on the intestine, we are even ready to attribute all our delayed or late defecation responses to such an action. We must insist, however, that the extraordinary prompt response to small optimum doses is due to central action, as such responses are inhibited by application of ergotamine tartrate to the medulla, by administration of large doses of morphine, and by simultaneous vagotomy and high transection of the cord.

6277

Relative Susceptibility of Adult and Young Mice to Asphyxiation.

R. C. AVERY AND J. M. JOHLIN.

From the Department of Bacteriology and Immunology and the Department of Biochemistry, Vanderbilt Medical School, Nashville.

It is generally stated that in carbon monoxide asphyxiation the smaller and the younger individuals, with the more active metabolism, tend to approach saturation more rapidly, and that small individuals therefore succumb sooner than large individuals. Four parts of carbon monoxide in 1000 parts of air are considered fatal to man after an exposure of less than one hour and presumably much more fatal to smaller animals such as mice.

Following the observation by one of us (R.C.A.) that the young of a family of wild mice survived while the parents died when exposed to illuminating gas, a series of experiments with 73 white mice was carried out.

The data in Table I show that, contrary to current belief, the younger survive longer. In pure concentrations of chemically inert gases (nitrogen, hydrogen and argon) the young survive exposures from 3 to 6 times as long as those which prove fatal to adults.

The lethal periods of exposure for adult and young mice, in the case of pure CO_2 and CO lie so close together that it is difficult to observe a survival of the young. This is partly due to the fact

TABLE I.

Gas	No. Adults	No. Young	Wt.	Time of Exposure	Results
			gm.	sec.	
Nitrogen	3		24-26	25- 40	Died during exposure
		3	3- 4.5	25-150	Survived
Argon	1		28	37	Died during exposure
		1	5	120	Survived
Hydrogen	3		22-28	18- 20	Died during exposure
		5	3- 5	18-135	Survived
		2	3.5-4	120	Died after exposure
CO_2	5		21-26	20- 36	Died during exposure
		5	3- 5.5	20- 33	Survived
		4	3.5-5	36- 60	Died after exposure
Illuminating Gas	2		22-23	18	Died during exposure
		3	4- 5	18- 40	Survived
CO	3		16-23	19- 22	Died during exposure
		4	3.5-6	19- 22	Died after exposure
	5		15-28	8- 15	Died after exposure
		3	2- 5	8- 12	Survived
0.4% CO	5		25-28		Died in 8, 19, 21, 42 and 60 min.
		3	5- 6		Died in 180, 225 and 255 min.
		4	1.5		Died in 270, 300, 315 and 360 min.
		4	2.5		Alive after 180 min., dead in 210 min.
	1		20	1 hr.	Survived
		4	2- 5	1 hr.	Survived

NOTE: In nearly every experiment shown in these data an adult and a young were exposed simultaneously in the same chamber.

that when adult and young animals were simultaneously exposed the gassing was continued until all muscle twitching in the animal which succumbed first had ceased. The young in such cases were still alive when exposure to the gas was discontinued but died in air within a few minutes after the termination of the experiment. When the time of exposure is sufficiently shortened the young survive indefinitely and the adults die immediately after removal from the gas.

The difference in time of survival between adult and young in illuminating gas is even more pronounced than in the case of pure CO.

In a 0.4% concentration of CO in air the time of exposure required to cause the death of the young is from 3 to almost 50 times as long as that necessary to cause the adult to die. In 0.4% CO 2-day-old mice similarly will outlive some which are 7 days old.

It was found that in no instance does a young animal die during one hour's exposure to 0.4% CO, which concentration is considered fatal to man for that period. In but one instance did an adult mouse survive for an hour under these conditions.

It developed in our experiments with the various gases, that in no instance, when young and old were exposed simultaneously, did the adult survive the young.

No determinations of the comparative blood CO pictures of these animals under conditions of these experiments have been made. It is, therefore, not possible to offer any physiological explanation of this survival phenomenon.

6278

Migration of Anthocyan Pigment in Plant Cells During the Flow of Electric Current, and Reversal by Acids and Alkalies.

L. R. BLINKS

From the Laboratories of The Rockefeller Institute for Medical Research

Visible effects of the flow of direct current through the cells of *Tradescantia* and other plants bearing anthocyan indicators have been known since the studies of Kühne.[1] They are generally accepted[2, 3, 4] as evidence of a change of acidity at the opposite ends of each cell, in accordance with the well known behavior of these pigments as indicators. A repetition of such experiments, with a variety of plants (from about a dozen families), leads the writer to a different interpretation, namely, a migration of the pigment within the cell.

The typical results with one group of plants including *Tradescantia (Zebrina)*, *Commelina*, *Rhoeo* (see also second group), *Lippia*, *Plantago*, *Sonchus*, beet and turnip, are as follows:

1. With uninjured cells, distant electrodes making contact to the tissue through agar, and low applied potential (1.5 to 6 volts in the epidermal strips as used) there is no apparent effect of current flow, the pigment remaining uniformly distributed throughout the cells and unchanged in hue.

2. With higher potentials (10 to 20 volts, probably causing some injury), or with low potentials in cells injured by chloroform or otherwise, there is a marked migration of pigment in most cells of

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[1] Kühne, W. Untersuchungen über das Protoplasma und die Contractilität. Leipzig, 1864, pp. 98-100.

[2] Bethe, A. *Arch. ges. Physiol.*, 1916, **163**, 147.

[3] Mast, S. O. *Z. vergl. Physiol.*, 1931, **15**, 309.

[4] Marsh, G., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 666.

the tissue toward the positive pole (anode). This results in concentrating it at the anodal end of each cell, the pink pigment there becoming deeper red in appearance, and depleting it at the cathodal end. The latter becomes almost clear, or more usually pale green, the general hue of the tissue when not pigmented with anthocyan. This green seems to have been taken to be due to increased alkalinity, but the expected intermediate hues, purple and blue, are not seen and the green is always pale.

3. Moreover the same (anodal) migration of pigment occurs in cells which have been immersed in ammonia, or other alkali. The pigment, now turned purple, blue, or dark green, depending on the degree of alkalinity, still becomes concentrated toward the anode and depleted toward the cathode in each cell, with no change of hue but only of density.

4. In almost all of these plants the direction of migration can be reversed by immersion of the tissue in acid solutions. The pigment becomes bright red or orange, and now migrates toward the cathode in each cell, becoming depleted toward the anode. The point at which this reversal occurs differs with different plants, and in one *(Lippia)*, migration remained anodal even after long immersion in 0.1 N HCl. In others the apparent "iso-electric point" of the pigment (or of some substance associated with its migration) was at pH 2, 3 or more.

5. The effects remain reversible for some time in the more favorable plants *(Commelina)*, as respects the color shifts by alternate immersion in acid or base, and the reversal of movement in the field with reversal of electrode polarity. In general the higher the current density, the faster the migration and the more densely crowded becomes the pigment at the end of each cell. The pigment migrates slowly back on breaking the current, or rapidly on its reversal.

6. Even after the pigment has been changed to a yellow or brown color by long exposure to the air, the migration may persist for a time, almost always anodally, although more sluggishly and with a marked flow of granules. The entire protoplast also contracts slightly toward the anode at this stage, although the nucleus usually remains stationary.

In contrast, a second group shows a cathodal migration of pigment without further treatment with acid. Rhubarb, with normally very bright acid cells is a logical example. In other plants, as *Ricinus, Euphorbia peplus, Bryophyllum, Melilotus, Stenotaphrum* (crab-grass), banana leaf and sometimes in *Rhoeo,* there is a cathodal migration even though the color of the pigment is pink or purple, indicating no great acidity. However, often without further treatment than exposure to the air in *Ricinus* (also in *Rhoeo* when initially cathodal), and in the others with ammonia, anodal migration is easily induced. The iso-electric point of the substance concerned in migration may thus vary considerably in different species, from about neutrality up to a very acid region. Consequently the migration could easily be reversed during an experiment either by natural changes of pH in the cells (*e. g.*, by loss of CO_2) or by the diffusion of acid and alkaline products from electrodes. These may account for certain conflicting polarities given in the literature.

Begonia, oleander, *Solidago,* nasturtium stem, *Lantana, Salicornia* and *Sesuvium* constitute a third group in which the colored cells show no very definite migration of pigment in either direction. Possibly there is a different type of pigment cells, and much of the current may flow elsewhere, particularly in the latter 2, which are salt marsh plants.

None of these findings, of course, argues against a change of acidity due to current flow in other experiments, or even in these, though masked by the more conspicuous migration. The writer hopes shortly to present indirect evidence that such a change of acidity occurs in another case. Meanwhile a classic and apparently beautifully direct demonstration must be called into some question, as must the relevance of arguments based upon it.

6279

Notes on Toxicity and Pharmacology of Indium.

W. F. VON OETTINGEN

From the Department of Pharmacology, School of Medicine, Western Reserve University

Indium was discovered spectroscopically by Reich and Richter in Freiburg zincblende. Although widely distributed in minerals, it usually occurs only in very small quantities, and only recently Westbrook[1] worked out a process to isolate this element in larger quantities from crude zinc liquors which are residues from the manufacturing of sulphuric acid.

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[1] Westbrook, L. R., Am. Electrochem. Soc., 57th General Meeting, St. Louis, May 29-31, 1930.

Indium is a white, lustrous metal, very soft and ductile and slightly heavier than zinc. It melts at 155° and boils at 1450°. It does not tarnish at ordinary temperature, but oxidizes rapidly above the melting point, burning with brilliant violet flame at higher temperature.

Chemically it resembles zinc in some respects and aluminum and iron in others. It is trivalent in stable compounds, and its sulphate forms alums with monovalent metal sulphates. Most indium salts are colorless, very soluble in water and hence do not crystallize easily. Alkalies precipitate the white indium hydroxide from the aqueous solutions of the salts, which precipitate may be redissolved by sodium and potassium hydroxide at room temperature, but it is reprecipitated at boiling temperature.

Preparation of Indium Solution. The material, for which we are indebted to the Grasselli Chemical Co., was stated to contain 268 gm. of indium chloride per liter. From this a tenth molar solution was prepared, containing 25.1 gm. of indium chloride per liter. This solution was found to have a pH of 1.8, using thymol blue as indicator. Attempts to neutralize this solution by means of alkali were not successful, because near the neutral point the hydroxide was precipitated. The mixture of indium chloride and sodium citrate, using 5 cc. M/10 indium chloride and 10 cc. M/10 sodium citrate can, however, be adjusted by means of alkali to pH 7.2 to 7.4.

Precipitation of Proteins. Two cc. of a 10% solution of fresh egg white in water were mixed with 1, 2, 3, 4, and 5 cc. of a 4% indium chloride solution, but gave no precipitate neither immediately after mixing nor after 24 hours. Similar experiments performed with 25% solutions of indium chloride showed precipitation only after some hours. Since these solutions were distinctly acid, they had to be compared with solutions of hydrochloric acid of the same pH. It was found that hydrochloric acid forms less precipitate after 24 hours than the 25% solution of indium chloride, from which it appears that the latter has some precipitant action on egg albumen.

Astringent Action on Mucous Membrane. This was studied by determining the filtration velocity through frog skin, according to Sollmann.[2] The following solutions were compared: 4% solution of indium chloride, diluted hydrochloric acid of the same pH, 4% indium chloride buffered with sodium citrate and alkali (pH 7.4), and sodium citrate of the same concentration.

After 4 hours, 4 cc. of the indium chloride solution, 7.9 cc. of the citrated indium chloride, 12 cc. of the diluted hydrochloric acid, and 18.2 cc. of the sodium citrate solution had passed the membrane. This shows that indium chloride, and less so, the citrated compound, have some astringent action.

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[2] Sollmann, T., *J. Pharm. Exp. Ther.*, 1922, **21**, 200.

Effect on the Fermentation by Yeast. This was studied by the method described by Sollmann and Pilcher,[3] using a 4% emulsion of yeast in 10% sugar solution. Five cc. of this emulsion were mixed with equal volumes of 0.5, 1, and 2% indium chloride solution buffered with sodium citrate and alkali, using for comparison equal concentrations of sodium citrate. These experiments showed no inhibition of the fermentation, and also series, in which unbuffered indium chloride and diluted hydrochloric acid of the same pH were used, showed only a very moderate inhibition, so that it appears that indium chloride has no marked antiseptic action.

Toxicity. The toxicity was first studied with earthworms, using the method described by Sollmann.[4] It was found that 4, 2, and 1% solutions of indium chloride buffered with sodium citrate and alkali, and also the corresponding concentrations of sodium citrate alone were fatal within from 5 to 15 minutes. Concentrations of 0.5 and 0.25% and corresponding concentrations of sodium citrate were distinctly toxic, whereas concentrations of 0.1 and 0.05% had no visible effect. Since practically identical results were obtained with sodium citrate solutions, with and without indium chloride, it appears that indium in form of its double salt is probably not more toxic for earthworms than sodium citrate.

The toxicity for the frog seems also to be very moderate, because after the subcutaneous injection of 2 cc. of 4% buffered indium chloride-sodium citrate mixture, the animals showed no other symptoms than those caused by sodium citrate alone. Injections of 2 cc. of a 4% indium chloride solution into the lymph sac had no effect for 24 hours, perhaps on account of precipitation of the material at the site of the injection.

The toxicity for mice is rather great (Table I).

From this table it appears that with subcutaneous administration the minimal fatal dose for mice is about 0.06 gm. indium citrate per kg. and that death may occur with a considerable delay. Oral administration appears to be less toxic. Mice stood daily feeding of a total of 24 gm. of indium hydroxide per kg. for 2 weeks, without apparent toxic effect.

The toxicity for rabbits is apparently higher, as indicated by the

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[3] Pilcher, J. D., and Sollmann, T., *J. Lab. and Clin. Med.*, 1924, **10**, 103.

[4] Sollmann, T., *J. Pharm. Exp. Therap.*, 1918, **12**, 129.

TABLE I.
Toxicity of Citrated Indium Chloride for Mice after Subcutaneous Injection.

	Weight			Days after Injection						
No.	Gm.	cc. inj.	Gm. per Kg.	1	2	3	4	5	6	7
1 cc. = 0.215 Gm. citrated Indium chloride.										
I	29	0.1	0.74		+					
II	27	0.2	1.6	+						
III	32	0.4	2.69	+						
IV	28	0.6	4.6	+						
V	25	0.8	6.88	+						
1 cc. = 0.0215 Gm. citrated Indium chloride.										
VI	26	0.1	0.083						+	
VII	24	0.2	0.179				+			
VIII	19	0.4	0.452				+			
IX	20	0.6	0.645			+				
X	21	0.8	0.818			+				
1 cc. = 0.01075 Gm. citrated Indium chloride.										
XI	20	0.1	0.054				survived			
XII	20	0.2	0.107							
XIII	22	0.4	0.195							
XIV	23	0.1	0.047							+
XV	23	0.2	0.094							+
XVI	21	0.4	0.205							+
XVII	20	0.1	0.054				+ 11th day			
XVIII	27	0.2	0.08				+			
XIX	22	0.4	0.196					+		
1 cc. = 0.0043 Gm. citrated Indium chloride.										
XX	19	0.2	0.045				survived			
XXI	18	0.25	0.055				8th day killed moribund			
XXII	28	0.5	0.077				survived			

results of E. E. Ecker, with subcutaneous injections of indium citrate in syphilitic rabbits.

Systemic Action. On account of the low pH of pure solutions of indium chloride and of the presence of sodium citrate in the buffered solutions, the effect of indium salts on isolated organs could not be studied. Some information could only be obtained by the subcutaneous injection of the citrated material into rabbits. The animals were prepared under local anesthesia for the registration of blood-pressure and respiration, and 0.54 gm. per kg. of indium citrate were injected subcutaneously. There was no visible effect during the first 7 hours. After this time, there was a slow but steady fall of the blood pressure. The amplitude of the heart beat was increased, showing a distinct diastolic tendency; as the fall of the blood pressure became more marked, the respiration was stimulated until the heart stopped suddenly in diastole. These animals showed no gross pathological changes.

Pathological Picture. Mice treated with subcutaneous injections of buffered indium citrate solutions showed a more or less marked depression shortly before death, the hind legs were paretic, the respiration rapid and spasmodic, later convulsions occurred and the

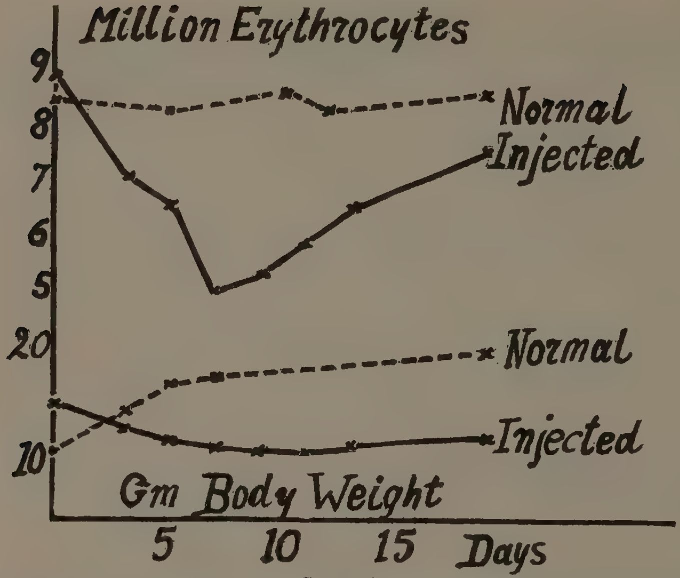

CHART 1.

Changes of the blood-count (upper diagram) and variations of body weight (lower diagram) in young mice after a single subcutaneous injection of indium citrate (0.06 gm. per kg.).

animals died from asphyxia. Animals treated with subfatal doses developed some anemia (Chart 1); and lost in weight, but recovered. With the same and even larger doses of zinc chloride prepared in the same way as the indium chloride solutions, several mice remained normal for several weeks.

Dr. H. Goldblatt examined microscopically the organs of some of the poisoned animals. The animals studied showed marked nephrosis and parenchymatic degeneration of the liver, but no pathological changes of the other organs, especially no edema or exudates. The site of the injection of all treated animals showed more or less marked induration which occasionally passed into necrosis.

Clinical Application. On account of the comparatively high atomic weight of indium (114.8), it was thought that indium iodide might be useful as contrast agent for fluoroscopy, but examination of the absorption of roentgen rays in the ionization chamber, performed by Dr. O. Glasser, showed that the absorption was in the same range as that of sodium iodide solutions of corresponding concentrations.

The resemblance of certain chemical properties of indium to those of bismuth suggested its trial as antisyphilitic agent. The citrated solution of the chloride, an oily suspension of the hydroxide, and an aqueous suspension of the metal are being tested in this respect by Dr. E. E. Ecker. Although these studies are not yet completed, they appear to indicate that indium in these forms has no curative effect on luetic lesions in rabbits.

6280

Biological Characteristics of Ovary-Stimulating Extracts Made from Blood of Pregnant Women*.

C. F. FLUHMANN

From the Department of Obstetrics and Gynecology, Stanford University School of Medicine

The demonstration that ovary-stimulating extracts prepared from human placenta (Collip *et al.*[1]), or from urine of pregnant women (Evans, Meyer, and Simpson[2]) differ from anterior pituitary gland implants in their influence on the weights of immature rat ovaries, is of considerable biological interest. It seemed of importance, therefore, to determine if extracts made from the blood of pregnant women were similar in this characteristic.

A crude estrin-free extract was prepared from blood by a method developed with the assistance of Dr. M. L. Tainter of the Department of Pharmacology, based on that of Wallen-Lawrence and Van Dyke[3] for the extraction of "hebin" from anterior pituitary glands. Forty to 50 cc., obtained by venapuncture, from each patient was placed in a receptacle containing a few crystals of sodium citrate, centrifuged, the red blood cells discarded, and the resultant clear blood plasma shaken up 5 times with ether. Ten volumes of 95% alcohol were then added to the blood plasma, the solution centrifuged, and the resultant precipitate washed twice with ether, dried, powdered, and extracted over night with 20 to 30 cc. of an isotonic sodium acetate-acetic acid buffer solution having a

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*Supported by a grant from the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Collip, J. B., Thomson, D. L., McPhail, M. K., and Williamson, J. E., *Can. M. A. J.*, 1931, **24**, 201.

[2] Evans, H. M., Meyer, K., and Simpson, M. E., *Am. J. Physiol.*, 1932, **100**, 141.

[3] Wallen-Lawrence, Z., and Van Dyke, H. B., *J. Pharm. and Exp. Therap.*, 1931, **48**, 93.

pH of about 4.4. The next morning it was centrifuged and to the clear supernatant solution was added 6 volumes of 95% alcohol. A white precipitate resulted which was recovered by centrifugalization, again washed with ether, dried and powdered. This powder contained the active element and was fairly readily dissolved in small amounts of the buffer solution for injection. It was generally so made up that 1 cc. of the extract was equivalent to 2.3 cc. of blood plasma and various dilutions were prepared from this stock solution.

Collip and his co-workers state that the injection of large doses of A.P.L. placental extract could not stimulate the ovaries of immature rats to develop to a size beyond that reached in adult life, while Evans, Meyer, and Simpson found that with prolan a curve in which ovarian weight is plotted against dose level quickly reaches a plateau and that a further 5-fold or even 10-fold increase in dose did not cause a further increase in ovarian weights. On the basis of the present preliminary work involving the use of over 80 immature rats it may be stated that the same characteristic can be ascribed to pregnancy blood extracts which readily induce the development of graafian follicles and lutein bodies. As shown in Table I, it has been impossible to produce any great increase in ovarian weight over a period of 5 days even by increasing the dose 50 times. This is in startling contrast to the tremendous increase in ovarian weights produced by anterior pituitary implants, as originally reported by Aschheim and Zondek and Smith and Engle.[4]

TABLE I

At end experiment		Duration experiment days	Amount injected	No. of injections	Wt. ovaries	Wt. uterus + tubes
Age days	Body wt.					
27	36.0	....	....	....	.013	.054
27	40.0	5	0.024	6	.009	.089
27	40.0	5	0.024	6	.011	.110
26	35.0	....	....	....	.015	.032
26	35.0	5	0.05	5	.024	.114
26	34.0	5	0.05	5	.018	.118
26	31.0	....	....	....	.008	.026
26	29.0	5	1.25	5	.012	.202
26	29.0	5	1.25	5	.014	.188
50	70.0	....	....	....	.016	.054
50	62.0	20	0.33	33	.020	.253
50	55.0	20	0.33	33	.023	.293
54	79.0	....	....	....	.014	.049
54	68.0	24	0.41	41	.049	.360
54	50.0	24	0.41	41	.033	.344
26	48.0	5	3 rabbit pituitary implants		.104	.139
26	47.0	5	“		.069	.128
26	49.0	5	“		.077	.094

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[4] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, **40**, 159.

Of considerable interest, however, is the pronounced increase in weight shown by the uterus under the influence of this extract. These weights are out of all proportion to the ovarian response and show a progressive increase according to the amount of extract administered. This is also marked even with small doses when the preparation is given over a prolonged length of time. In the appended table are a few examples illustrating the effects obtained with one of the extracts, and for comparison are listed the findings in 3 immature rats each of which received one rabbit anterior pituitary gland implant daily for 3 days. However, as pointed out by Smith and Engle,[4] the uterine weights do not represent the absolute tissue gain as they were almost all done at a stage of uterine engorgement when the vaginal smear showed cornified or nucleated epithelial cells. The factors concerned in this phenomenon are not clear, but it is believed that the uterine growth is not due to ovarian hormones present in the extracts since these were ineffective in immature spayed rats.

6281

Observations on Mørch's Method for Standardization of Thyroid Gland Preparations.

W. W. PALMER AND J. P. LELAND.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

Mørch[1] devised a method for the standardization of thyroid preparations based on the percentage increase of the carbon dioxide output of white mice after administration of thyroid preparations. Gaddum and Hetherington[2] compared the physiological activity of thyroid preparations with the thyroxine content as determined by Harington and Randall.[3] It seemed desirable to investigate the question whether the thyroid glands of patients with exophthalmic goiter contained some substance other than thyroxine itself which might cause an increase in the basal metabolic rate. An answer seemed possible by feeding animals equi-thyroxine doses of different gland preparations obtained from patients with Graves' disease and from normal individuals. If the physiological activity of the 2 types of preparations proved to be the same, this might be considered evidence that the increase in basal metabolism characteristic of Graves' disease could be accounted for by the thyroxine content alone. It would then be unnecessary to postulate the presence of any other toxic substance. The method developed by Mørch was chosen since it has the necessary feature that small amounts of material can be employed.

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[1] Mørch, J. R., *J. Physiol.*, 1929, **67**, 221.

[2] Gaddum, J. H., and Hetherington, M., *Quart. J. Pharm. and Pharmacol.*, 1931, **4**, 183.

[3] Harington, C. R., and Randall, S. S., *Quart. J. Pharm. and Pharmacol.*, 1929, **2**, 501.

Normal thyroid preparations from the series of Leland and Foster[4] and exophthalmic goiter preparations from the series of Gutman, Benedict, Baxter and Palmer[5] were used. All glands were analyzed by the method of Leland and Foster. The optimal experimental conditions worked out by Mørch were closely adhered to and it was found that the carbon dioxide output of the mice could be determined with the degree of precision which he claims (Table I).

TABLE I.
Normal Mice.

No.	Wt. in gm.	Production of CO_2 in 24 hours on consecutive days in gm.					Average
		1st	2nd	3rd	4th	5th	
11	18.0	3.14	2.90	2.99	3.13	3.13	3.06
12	17.0	3.10	3.20	3.24	3.10	2.94	3.12
17	17.5	3.36	3.45	3.47	3.44	3.34	3.41
29	17.5	3.44	3.60	3.43	3.35	3.39	3.44
20	17.0	3.14	3.13	3.23	3.20	3.25	3.19
25	17.0	3.55	3.58	3.74	3.74	3.63	3.65
31	18.5	3.27	3.35	3.38	3.44	3.34	3.36

The commercial thyroid preparation used as a standard (Burroughs Wellcome & Co.) was first administered by mixing dry with the food as directed by Mørch. Later it was given in the form of a uniform suspension in 0.9% NaCl. A sufficient amount of the thyroid preparation was weighed out so that 2 cc. contained the correct dosage for a 20 gm. mouse. Daily variation of the dosage with change in weight was thus made easy. The suspension was shaken well and delivered directly onto the dry food, followed by 3 cc. of milk. The food intake was cut to a minimum for weight maintenance, so that every morsel was eaten. This method of administration seemed more satisfactory than mixing a very small amount of gland material with a large quantity of food. No other variations were made from Mørch's technique.

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[4] Leland, J. P., and Foster, G. L., *J. Biol. Chem.*, 1932, **95**, 165.

[5] Gutman, A. B., Benedict, E., Baxter, B., and Palmer, W. W., in press.

The results are shown in Table II.

TABLE II.

Mouse No.	Thyroid Preparation	Type Gland	Total Iodine	Thyroxine Iodine	CO$_2$ Increase Av. 5 days	Daily Dosage per 20 gm. Mouse		Control Response to Standard Preparation (2)
						Thyroid	Thyroxine Iodine	
			mg. per gm.	mg. per gm.	%	mg.	γ (1)	
1	B. W. & Co.	Hog	2.54	0.592	—1.2	0.12	0.071	
2	”	”	2.54	0.592	6.7	0.12	0.071	
3	”	”	2.54	0.592	7.4	0.12	0.071	
4	”	”	2.54	0.592	8.0	0.15	0.089	
5	”	”	2.54	0.592	—1.8	0.15	0.089	
6	”	”	2.54	0.592	9.9 (3)	0.20	0.118	
7	”	”	2.54	0.592	25.5	0.20	0.118	
8	”	”	2.54	0.592	22.6 (3)	0.20	0.118	
9	”	”	2.54	0.592	8.6	0.25	0.148	
10	”	”	2.54	0.592	29.6	0.25	0.148	
11	”	”	2.54	0.592	22.3	0.25	0.148	
12	N. J.	Graves’	2.80	0.540	21.3	0.274	0.148	
13	”	”	2.80	0.540	1.2	0.274	0.148	2.3
14	No. 40	Normal	1.43	0.352	—3.3	0.420	0.148	1.1
15	”	”	1.43	0.352	4.0	0.420	0.148	10.3
16	”	”	1.43	0.352	3.6	0.420	0.148	9.9
17	”	”	1.43	0.352	2.6	0.420	0.148	
19	P. S.	Graves’	3.90	1.10	18.7	0.134	0.148	
20	”	”	3.90	1.10	8.1	0.134	0.148	
21	A. S.	”	3.18	0.468	0.8	0.316	0.148	—5.2
22	”	”	3.18	0.468	1.9	0.316	0.148	7.4
23	C. W.	”	1.54	0.16	—3.4	0.92	0.148	—2.4
24	”	”	1.54	0.16	—10.0	0.92	0.148	1.3
25	”	”	1.54	0.16	7.1	0.92	0.148	4.7
26	”	”	1.54	0.16	1.6	0.92	0.148	14.6
27	”	”	1.54	0.16	8.7	0.92	0.148	10.7
28	”	”	1.54	0.16	—2.4	0.92	0.148	7.9
29	A. H.	”	2.14	0.535	—7.9	0.277	0.148	0.4
30	”	”	2.14	0.535	—15.3	0.277	0.148	—14.2
31	”	”	2.14	0.535	2.1	0.369	0.148	12.4
32	A. M.	”	3.88	0.69	15.0	0.213	0.148	
33	”	”	3.88	0.69	14.9	0.213	0.148	
34	No. 27	Normal	1.21	0.272	—0.3	1.09	0.296	
35	”	”	1.21	0.272	7.9	1.09	0.296	
36	”	”	1.21	0.272	17.2	1.09	0.296	
37	”	”	1.21	0.272	16.5	1.09	0.296	
38	”	”	1.21	0.272	13.1	2.18	0.592 (4)	

(1) 1 γ = 0.000001 gm.

(2) Animals failing to show a significant increase in CO$_2$ production after treatment with human thyroid preparations were rested from 10 to 15 days and then treated with the same dose of standard preparation.

(3) Accidental death after 2 days in the respiratory chamber.

(4) Two animals (not reported) died after 3 weeks' treatment with this dosage.

Although Gaddum and Hetherington found it necessary to use large amounts of thyroid in order to obtain significant increases in CO$_2$ production, positive responses were obtained in this laboratory in some cases at the low levels of dosage recommended by Mørch.

Gaddum and Hetherington attribute this difference in dosage to the possibility that their animals were less sensitive than those of Mørch. From Table II it may be seen that no valid conclusions can be drawn as to the comparative activity of different thyroid preparations since different mice vary markedly in their response to the same dose of a given thyroid preparation, even though administered in an identical manner to animals kept under similar conditions. For example, a given dose of gland No. 27 produced changes in CO_2 output ranging from —0.3 to +17.2, while preparation marked N. J. produced in one case +21.3% and in another case +1.2%.

Our experience with the method of Mørch is disappointing. Possibly observations sufficiently great in number to afford a comparison of the means rather than of individual values might yield intelligible results. The method is long and tedious. Its reliability, even with a large series, is doubtful.

6282

Assay of Thyroid by Chemical Estimation of the Thyroxine Content.

A. B. GUTMAN,* E. M. BENEDICT AND W. W. PALMER.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

To investigate the variation in the thyroxine content of commercial thyroid preparations, thyroid tablets from 15 representative pharmaceutical firms were obtained in the open market and analyzed for total iodine, inorganic iodine and thyroxine. Harington and Randall[1] made a similar survey, and found in tablets "each equivalent to 5 grains of fresh thyroid gland", a variation of about 600% in the thyroxine content, as estimated by the total iodine in the acid-insoluble fraction. However, recent work[2, 3, 4] suggests that absolute thyroxine values based on this method may be too high since after 4 hours' hydrolysis with N/1 NaOH the acid-insoluble fraction appears to consist of a heterogeneous mixture of free thyroxine and incompletely hydrolyzed polypeptides containing diiodotyrosine. It was therefore thought desirable to follow a procedure which we had found satisfactory for the iodine partition of pathological thyroid glands.[4] This is based on the Leland and Foster method for the estimation of thyroxine,[3] a modified Kendall method for the determination of total iodine[5] and aqueous extraction of the desiccated gland for estimation of inorganic iodine. All determinations were done in duplicate, the mean discrepancy between duplicates in the thyroxine determinations being 2.7%.

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* George Alexander Fellow of the Leopold Schepp Foundation

[1] Harington, C. R., and Randall, S. S., *Quart. J. Pharm. and Pharmacol.,* 1929, **2**, 501.

[2] Kendall, E. C., and Simonsen, D. G., *J. Biol. Chem.*, 1928, **80**, 357.

[3] Leland, J. P., and Foster, G. L., *J. Biol. Chem.*, 1932, **95**, 165.

[4] Gutman, A. B., Benedict, E. M., Baxter, B., and Palmer, W. W., *J. Biol. Chem.*, in press.

Fifteen different products were found to have the following thyroxine iodine contents, in terms of mg. per one grain tablet. .0099, .0124, .0187, .0226, .0257, .0260, .0270, .0280, .0291, .0305, .0312, .0323, .0325, .0329, .0355. The difference between the minimum and maximum values observed is .0256 mg., a variation of 259% with respect to the minimum figure.

The variations in the physiological activity of commercial thyroid preparations as indicated by biological assay[6, 7, 8] and in the thyroxine content as suggested by the data of Harington and Randall and ourselves, seem to be inconsistent with present therapeutic standards. Adherence to U.S.P. requirements markedly reduces the variation in thyroxine content. However, of the 15 different preparations examined, only 7 contained "not less than 0.17 and not more than 0.23% iodine in thyroid combination" if iodine in thyroid combination is defined as total iodine; while only 6 complied with the requirements, if it is defined as organic iodine. The thyroxine iodine content in mg. per grain tablet in the first group varied from 0.0187 to 0.0312, or 67%, while the thyroxine iodine content of the second group varied from 0.0260 to 0.0329, or only 26.5%. But while the variation in thyroxine content thus appears to be adequately controlled by keeping the organic iodine content within U.S.P. limits, the possible variation is much greater than that actually observed in our series. A preparation with an organic iodine content of 0.17% may have a thyroxine iodine percent of organic iodine of 18%, and another preparation with an organic iodine content of 0.23% may have a thyroxine iodine percent of organic iodine of 35%. Both preparations would satisfy the U.S.P. requirements, but the variation in the thyroxine content of the 2 preparations would be almost 175%. The possibility of adulteration must also be considered. Addition of a cheap, water-insoluble, relatively stable substance such as iodized casein would be very difficult to detect by chemical means if determination of organic iodine or of the acid-insoluble iodine fraction is used for assay.

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[5] Foster, G. L., and Gutman, A. B., *J. Biol. Chem.*, 1930, **87**, 289.

[6] Munch, J. C., *Bioassays*, 1931, 665.

[7] Hunt, R., *Arch. Int. Med.*, 1925, **35**, 671.

[8] Lipschitz, W., and Girndt, O., *Arch. f. exp. Path. u. Phar.*, 1931, **159**, 259.

Standardization of thyroid by direct determination of the thyroxine content after the method of Leland and Foster appears to be the most satisfactory solution of the problem. The determinations can easily be made in cooperating commercial laboratories, an important consideration for any practical method. By maintaining the thyroxine content of thyroid tablets within specified limits, one of the most important of the variables encountered in thyroid medication may be eliminated.

6283

Retardation of Tuberculous Infection in Guinea Pigs Vaccinated with Killed Tubercle Bacilli as Shown by Cultural Method.

JULES FREUND.

From the Henry Phipps Institute, University of Pennsylvania.

The effect of vaccination with killed tubercle bacilli upon the resistance of animals to tuberculous infection has been studied extensively. It has been repeatedly shown that guinea pigs can be sensitized by this means to the intracutaneous injection of old tuberculin (Römer,[1] Bessau,[2] Zinsser, Ward and Jennings,[3] Petroff[4]). Its influence upon the course of subsequent infection, however, has been studied with less conclusive results under widely varying experimental conditions. Römer,[1] Zinsser, Ward and Jennings,[3] Petroff,[4] and L. Lange[5] conclude that such treatment gives some protection; Dold,[6] and Seligmann and von Gutfeld,[7] that it gives none. B. Lange and his associates[8] believe that inoculation with heat-killed tubercle bacilli affords only a very slight protection, which can be as readily obtained with killed *B. coli* or a lipoid derived from animal tissue ("Helpin"). In the work thus far reported, the effect of immunization has been estimated by comparing in vaccinated and unvaccinated animals the duration of disease and the pathological changes observed at autopsy.

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[1] Römer, P. H., *Beitr. klin. Tub.*, 1909, **12**, 185.

[2] Bessau, G., *Berl. klin. Woch.*, 1916, 801.

[3] Zinsser, H., Ward, H., and Jennings, F. B., *J. Immun.*, 1925, **10**, 719.

[4] Petroff, S. A., and Stewart, *J. Immun.*, 1926, **12**, 97. Petroff, S. A., Branch, A., and Jennings, F. B., *Ibid.*, 1929, **16**, 233.

[5] Lange, L., *Zentralbl. Bakt.*, 1921, **85**, 26.

[6] Dold, H., *Klin. Woch.*, 1925, 1763.

[7] Seligman, E., and von Gutfeld, F., *Deitsche med. Woch.*, 1925, 1064.

[8] Lange, B., and Freund, R., *Zeitsch. Hyg.*, 1926, **105**, 571. Lange, B., and Jochimsen, E., *Ibid.*, 1927, **107**, 426. Lange, B., Jochimsen, E., and Magat, J., *Ibid.*, 1927, **107**, 645.

Tubercle bacilli injected into the tissues of guinea pigs penetrate the lymph ducts and cause formation of tubercles in the nearest lymph nodes, and the rate of spread of the bacteria in the regional lymphatic system is an important factor in the progress of the disease. Opie[9] has shown that soluble antigens such as crystalline egg albumin and horse serum, spread rapidly from the site of injection and enter the blood stream in normal animals, but that when rabbits have been immunized by repeated injections these antigens are fixed at the site of the acute local inflammation (Arthus phenomenon). Similarly Willis[10] has shown a delay in the dissemination of tubercle bacilli along the lymphatics draining the site of injection in guinea pigs immunized with *living avirulent* tubercle bacilli. They demonstrated the presence and estimated the quantity of bacilli in the organs by guinea-pig inoculation. The present study is an attempt to determine by the cultural method, whether inoculation with *heat-killed* tubercle bacilli retards the spread of living tubercle bacilli in the regional lymphatics.

Experimental. The vaccine was prepared from a very virulent strain of tubercle bacillus of human type (489 A). Three-weeks-old cultures removed from glycerin agar were dried over sulphuric acid *in vacuo*. One cc. of the suspension contained 2 mg. of tubercle bacilli (dry weight). The suspension was sealed in ampules and immersed in boiling water for 20 minutes. Six guinea pigs injected with 5 cc. of the vaccine were found free of tuberculosis when killed 6 months later. No growth was obtained on Dorset's medium.

Male guinea pigs weighing from 350 to 420 gm. were used. One cc. of vaccine was injected close under the skin about 1 cm. below the right clavicle three times 5 days apart. One month later, 0.5 cc. vaccine was similarly injected. One or two abscesses were found at the site of injection in all vaccinated animals. Five days after the last vaccination 6 of the vaccinated guinea pigs were injected with 0.1 cc. of a 1-in-10 dilution of old tuberculin. All of them showed tuberculin reactions with necrosis. This skin reaction differed in one respect from those seen in tuberculous guinea pigs: there was relatively more necrosis and less edema. Five days later the vaccinated and control (not vaccinated) animals were infected with tubercle bacilli. The strain used was of human type (489 A); 0.00001 mg. killed guinea pigs within 6 months when injected subcutaneously. Two-tenths cc., containing 0.001 mg. of tubercle bacilli, was injected into the skin of the left groin of 3 vaccinated and 3 control guinea pigs, alternately. At short intervals guinea pigs of both groups were killed, the organs removed and cultured, and the macroscopic pathological changes noted. From some of them (Table I) one-half of the spleen was cultured. The following procedure was employed in culturing.

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[9] Opie, E. L., *J. Exp. Med.*, 1924, **39**, 659.

[10] Willis, H. S., *Am. Rev. Tub.*, 1925, **11**, 427.

The guinea pigs were bled to death and the organs were removed aseptically, cut into small pieces and ground with sea sand alone and with 1.2 cc. of phosphate solution. Of the resulting suspensions, some prepared from the spleen were treated with Petroff's sodium hydroxide method to kill microorganisms other than tubercle bacilli. The suspensions were placed in test tubes containing glass beads and shaken, and 0.1 cc. was spread on each of 3 or 4 tubes of Dorset's medium, and on 3 or 4 tubes of Petroff's medium. To prevent evaporation paraffin was poured on the cotton plugs from a coffee pot. The colonies were counted at various intervals, and the final reading was made during the fourth month of incubation.

The following tables show in what numbers and at what intervals after infection, colonies of tubercle bacilli were obtained from the lymph nodes and spleen of vaccinated and control animals.

Tables I and II show 2 distinct differences in the vaccinated and unvaccinated animals. (1) Positive cultures were obtained from the lymph nodes of the unvaccinated animals after a shorter period of infection. Unexpectedly, this order seemed to be reversed in the spleen, but this result was probably only apparent, and due to the fact that the colonies were obtained from the spleen of one vaccinated animal by the direct method, from the others, by the sodium hydroxide method, which yields fewer colonies. (2) A larger number of colonies were obtained from the control group.

These observations show that immunization of guinea pigs with heat-killed tubercle bacilli, having produced hypersensitiveness to old tuberculin, retards dissemination of living tubercle bacilli through the regional lymphatics. Evidence as to whether the retardation is due to a mechanical fixation, or to destruction (killing) of the injected tubercle bacilli, or to both, could probably be obtained by culturing the tissue injected with tubercle bacilli.

TABLE I.

Number of Colonies Obtained from Vaccinated and Unvaccinated Guinea Pigs after Intracutaneous Injection with 0.001 mg. Tubercle Bacilli.

Interval after infection	Abdominal-inguinal lymph node†		Inguinal lymph node		Iliac lymph node		Spleen	
	v.	c.	v.	c.	v.	c.	v.	c.
days								
1	0	12	—	—	—	—	0*	0*
8	0	60	—	—	—	—	60	0*
10	0	0	—	—	—	—	0	0*
13	0	2,000	—	0	0	0	0	0*
16	—	2,160	—	1,320	—	—	—	438
21	80	—	0	—	0	600	0	0
23	—	—	0	0	0	60	—	60
29	—	—	—	4,800	—	500	—	—
33	—	—	—	0	—	0	—	0
35	4,800	—	0	—	0	—	0	—
36	—	9,600	—	—	—	8,400	—	—
37	—	—	0	—	0	—	0	—
42	2,200	—	—	20	—	0	0	9,600
43	1,200	—	—	—	780	—	180	—
44	—	0	—	4,800	—	720	—	1,020
48	2,400	—	1,440	12,000	24,000	—	0	24,000
50	35,000	—	1,200	12,000	—	—	0	—
55	6,720	18,000	31,000	24,000	4,200	12,000	600	2,400
64	1,800	—	22,800	—	36,000	12,000	54,000	48,000
67	24,000	—	16,800	—	100,000	—	—	—

v. vaccinated with heat-killed tubercle bacilli.
c. control.
0 no colonies found.
— not done or lost on account of contamination.
* treatment with sodium hydroxide.
† Also called superficial inguinal lymph node.

TABLE II.

Immunization with Heat-killed Tubercle Bacilli.

Number of Days Elapsed Between Infection and Positive Cultures.

Organ	Vaccinated	Unvaccinated
Abdominal-inguinal lymph node	21	1
Inguinal lymph node	48	16
Iliac lymph node	43	21
Spleen	8	16

6284

Conjugation of Benzoic Acid with Glycine, a Test of Liver Function.

ARMAND J. QUICK. (With the technical assistance of Mary A. Cooper.)

From the Department of Surgical Research, Cornell University Medical College.

In a recent study on the conjugation of benzoic acid in man, the author found that the excretion of hippuric acid proceeded at a constant rate irrespective of the amount of sodium benzoate administered.[1] Since it was further found that the output of hippuric acid was greatly increased by feeding glycine or foods rich in glycine such as gelatine, it became obvious that the rate of hippuric acid formation is dependent upon the speed with which the body can synthesize glycine. It was determined that a normal adult can produce enough glycine to conjugate from 0.90 to 1.30 gm. of benzoic acid per hour.

Since the main site of the synthesis of glycine is presumably the liver, it was considered probable that certain types of liver damage might show a diminution in the hourly excretion of hippuric acid. On this basis a test was carried out as follows: 5.9 gm. of sodium benzoate dissolved in 150 cc. of water is administered 1 hour after a breakfast consisting of coffee and toast. Complete hourly specimens of urine are collected for 4 hours. Hippuric acid is determined by the author's method.[2] A simpler method which is sufficiently accurate for clinical purposes can be employed. In this procedure the hourly specimen is measured, transferred to a small beaker, and acidified with concentrated HCl until acid to Congo red. (1 cc. of the acid is usually sufficient.) The solution is vigorously stirred until the hippuric acid has completely crystallized out, and then is allowed to stand at room temperature for 1 hour. The hippuric acid is filtered off on a Buchner funnel or a small filter plate, washed with cold water, allowed to air dry, and finally weighed. (Weighing to the second decimal place is sufficiently accurate.) To obtain the total hippuric acid, one adds to the weight of the precipitated crystals, the calculated amount remaining in solution. One hundred cc. of urine will hold in solution approximately 0.33 gm. of hippuric acid. If the volume of any specimen exceeds 150 cc., it should be slightly acidified with acetic acid, and concentrated on a water bath to about 50 cc. before precipitating the hippuric acid.

In applying the test to various liver diseases, the most striking results were obtained in cases of toxic jaundice including catarrhal jaundice, and in obstructive jaundice of moderately long standing. In all these cases a definite diminution in the rate of hippuric acid excretion occurred, and significantly, the hourly output often remained low long after the jaundice had cleared up. In Table I a few typical results are recorded.

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[1] Quick, A. J., *J. Biol. Chem.*, 1931, **92**, 65.

[2] Quick, A. J., *J. Biol. Chem.*, 1926, **67**, 477.

TABLE I.
Hourly Excretion of Hippuric Acid.

Time	Normal			Obstructive Jaundice		Catarrhal Jaundice	
	I	II	III				
hr.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	1.29	0.43	0.81	0.54	0.43*	0.23	0.35*
2	1.96	1.96	1.46	0.93	0.56	0.51	0.51
3	0.82	1.28	1.51	0.93	0.54	0.65	0.44
4	0.28	0.79	1.49	0.81	0.70	0.54	0.69

* 10 days after the first test.

6285

Appearance of Large Amounts of Non-Stainable Cultivable Granules in Bacterial Cultures on Saccharose Media.

LOUIS DIENES.

From the Department of Pathology and Bacteriology, Massachusetts General Hospital, Boston.

A Gram-positive spore bearing aerobic bacillus, isolated following accidental contamination of a saccharose broth tube, was found to grow on a saccharose agar plate as a thick soft spreading film. This film later became of semi-liquid consistency and in it the bacteria could be seen whirling about. Using a dark background preparation, the culture consisted for the most part of scarcely perceptible minute granules, often arranged in large clumps. Masses of these granules were seen in saccharose broth cultures as well as upon solid media. Using a very strong light or sunlight with the dark field microscope, these granules were also visible in strong Brownian movement. They were not stained by the usual methods. They were made visible by the following technique: a loopful of a broth culture or of an emulsion of the agar growth was mixed on a slide with a loopful of a crystal violet solution (a 1:5 or 1:10 dilution of the solution used for Gram stain). About one minute later a loopful of a 5% solution of silver nucleinate was added and the whole mixture was spread over the slide as a thin film. A second method also gave satisfactory results: the emulsion was spread over the slide, dried, stained with crystal violet without fixing, washed, covered with silver nucleinate without previously being dried, and finally the silver solution was allowed to drain off by tilting the slide. The bacteria appeared violet, the granules colorless on a brown background.

When a thick streak from a well grown culture is made on a saccharose agar plate and kept at 25°-30°C. the growth begins to spread in 15-30 minutes. With the aid of a hand lens the bacterial mass is seen to be surrounded by a thin film which spreads laterally at a rate of 2-3 mm. in an hour. Microscopically this film does not consist of bacteria, but of an amorphous material arranged in zig-zag branching streams and containing many tiny colony-like thickenings which are often connected with each other though in other areas connections between the tiny colony-like agglomerations cannot be made out. An impression preparation of this film reveals only the non-stainable granules without bacteria. As the film becomes thicker, the bacteria move actively into it from the edge of their colony, but this bacterial invasion is soon followed by a further extension of the spreading film. This phenomenon occurs only on saccharose plates, and not on plain, dextrose or blood agar.

The edge of this spreading film was touched with the tip of a fine glass capillary and another part of the plate inoculated. With considerable frequency clusters of tiny round colonies consisting solely of the non-stainable granules without admixture of bacteria developed. Definite growth of the transplanted colonies could be observed.

The filtrate of a thoroughly centrifuged saccharose broth culture through a Berkefeld N filter is usually turbid and on microscopic examination contains no bacteria but a large number of non-stainable granules. The filtrate remains sterile for many days and transplants of it on agar or in broth have not grown. However, in some filtrates contaminated in the course of repeated transfers the contamination was accompanied by an enormous increase in the number of granules, and on one occasion a large number of granule colonies grew out on a saccharose agar plate between the contaminating coccus colonies. When the spores of the bacteria were heated at 100°C. for one minute, and inoculated into saccharose broth the non-stainable granules were reproduced. It seems necessary to conclude therefore that they are derivatives of the bacterium.

Oerskov[1] first described similar phenomena with a fluorescentlike bacterium isolated from milk. We found this organism without difficulty in several fresh milk samples and were able to confirm with it most of the observations of Oerskov. We failed, however, with the milk organisms to demonstrate the most important point, that a growth of the non-stainable granules can be obtained which is certainly independent of the growth of the bacteria, though the evidence for this is strongly suggestive.

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[1] **Oerskov, T.,** ***Zbl. f. Bakt.,*** **1931, 1, 120.**

It has long been known that upon saccharose media the colonies of many bacteria develop a peculiar slimy consistency. We have examined the slime produced by 6 different types of organisms grown on saccharose media and have found it in all cases to consist of particulate matter in the form of non-stainable granules, similar in all respects to the findings described above. It is of special interest that streptococci from human throats often were found to form large semi-transparent colonies on saccharose plates. The granules in these colonies are very small and could be seen only with dark field illumination, being apparently too small to show up with the staining methods described above.

6286

Colon Activation by Intravenous Hypertonic NaCl Injection in Unanesthetized, Trained Dogs.

FREDRICK F. YONKMAN. (Introduced by F. H. Pratt.)

From the Department of Pharmacology, Boston University School of Medicine.

Since Hughson and Scarff[1] first reported peristaltic augmentation of the ileum after NaCl intravenously in anesthetized cats, several investigators have confirmed the observation.[2, 3, 4, 5] Regarding the response of the colon no action has been experimentally demonstrated. Thus our results seem interesting since they show that the large intestine can be activated by salt solutions of adequate hypertonicity when intravenously administered.

This work was carried out in unanesthetized, trained collies, which had undergone cecostomy after the method described by Plant and Miller.[6] Rubber balloons of convenient size, under uniform water pressure were inserted into the anterior colon and registrations were recorded through a closed system[6] by means of Mendenhall's[7] modified Brodie[8] bellows. Varying amounts, 2 to 15 cc., of NaCl 5% and 20% concentration were injected intravenously and intracardially.

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[1] Hughson, W., and Scarff, J. E., *Johns Hopkins Hosp. Bull.*, 1924, **35**, 197.

[2] Ross, J. W., *Canad. Med. Assn. J.*, 1926, **16**, 241.

[3] Dreyer, N. B., and Tsung, Thelma, *J. Pharmacol. and Exp. Therap.*, 1929, **36**, 629.

[4] Orr, T. G., Johnstone, P. N., and Haden, R. L., *Surg. Gynec. and Obstet.*, 1931, **52**, 941.

[5] Reid, P. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 220.

Results. 5% NaCl only slightly (a) augments colonic tonus, (b) increases peristaltic amplitude.

5% NaCl does not alter peristaltic frequency.

5% NaCl shows little or no disturbance of frequency and amplitude of rhythmic waves.

20% NaCl definitely (a) augments colonic tonus, (b) increases peristaltic amplitude, (c) induces peristalsis, in the quiescent, atropinized colon.

20% NaCl shows little or no augmentation of frequency and amplitude of rhythmic waves.

Similar amounts of normal saline fail to affect the colon.

20% NaCl, 1.0 cc. per kg., as here administered did not produce vomiting or retching movements.

5% and 20% NaCl failed to produce defecation in these particular experiments.

No evident distress was ever observed in over 20 experiments.

Conclusions. Reasonably small amounts of hypertonic NaCl solutions intravenously injected promote or augment activity of the colon. Since this increased activity can be induced after atropinization, NaCl, in this instance, probably affects either Auerbach's plexus or the intestinal muscularis directly. We are not overlooking the possibility that sufficient disturbance of oxygen transport may occur to produce partial asphyxiation which may stimulate the intestinal musculature. This possibility is being investigated. The beneficial application of intravenous hypertonic NaCl in colonic stasis—myogenic or neurogenic in origin—seems worthy of consideration by the clinician.

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[6] Plant, O. H., and Miller, G. H., *J. Pharmacol. and Exp. Therap.*, 1926, **27**, 149.

[7] Mendenhall, W. L., Demonstration, Fed. Am. Soc. for Exp. Biol., Rochester, 1927.

[8] Brodie, T. G., *J. Physiol.*, 1901, **27**, 473.

6287

Presence of Carotenase in the Liver of the Dog.

ARTHUR C. PARIENTE AND ELAINE P. RALLI.

From the Laboratories of the Department of Medicine, The University and Bellevue Hospital Medical College, New York University.

That carotene is converted to vitamin A *in vivo* has been shown by Moore,[1] Capper and his co-workers,[2, 3] and by Drummond, Ahmad, and Morton.[4] Moore[5] postulated that this conversion takes place in the liver. Olcott and McCann[6] made a toluene water extract of the livers of A-deficient rats, and with this extract were able to convert colloidal carotene to vitamin A *in vitro*. They believed the activity of the extract to be due to an enzyme which they named carotenase.

We attempted to prepare carotenase from the livers of normal dogs and of dogs on A-deficient diets. We succeeded only once out of 4 trials. In the successful attempt, we noted that in preparing the carotenase, much of the blood had remained in the liver. It occurred to us that the blood buffers present might have been responsible for the preservation of the enzyme. We therefore made a liver extract, using a phosphate buffering solution of pH 7.4 instead of distilled water. The buffer was made by taking 39.5 cc. of 0.2 N (carbonate free) NaOH, 50 cc. of 0.2 M acid potassium phosphate, and this was made up to 200 cc. with distilled water. We found that such a solution always converted colloidal carotene to vitamin A as shown by a positive antimony trichloride reaction, even when watery extracts of the same liver did not. Colloidal carotene itself failed to give a positive antimony trichloride reaction. The liver extract solution also was tested but gave no reaction.

Olcott and McCann showed that the enzyme was inactive after boiling. We have noted that it is also destroyed by exposure to cold. The enzyme can be kept active by keeping it at 37°C.

Method. 30 gm. of liver was thoroughly ground with sand and

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[1] Moore, T., *Biochem. J.*, 1930, **24**, 692.

[2] Capper, N. S., *Biochem. J.*, 1930, **24**, 980.

[3] Capper, N. S., McKibbin, I. M. W., and Prentice, J. H., *Biochem. J.*, 1931, **25**, 265.

[4] Drummond, J. C., Ahmad, B., and Morton, R. A., *J. Soc. Chem. Ind.*, 1930, **49**, 291 T.

[5] Moore, T., *Biochem. J.*, 1931, **25**, 275.

[6] Olcott, H. S., and McCann, D. C., *J. Biol. Chem.*, 1931, **94**, 185.

Presence of Enzyme Carotenase in Buffered and Unbuffered Liver Extract Solutions.

Dog	Previous Diet	Carotenase Unbuffered	Buffered
40	Normal	negative	
61	A-deficient diet	negative	
51	A-deficient diet	negative	
41	Normal	positive	
12	A-deficient diet plus carotene	negative	positive
65	A-deficient diet	positive	positive
	after 4 days' incubation at 37°C.	faintly positive	positive
42	Normal	negative	positive
	after 4 days' incubation at 37°C.	negative	positive

75 cc. of the above buffering solution saturated with toluene was added. The mixture was allowed to incubate at 37°C. for 24 hours and then filtered. Two cc. of the filtrate were added to 6 cc. of a colloidal solution of carotene* containing approximately 0.02 mg. of carotene per cc. The latter was prepared according to the method of Fodor and Schoenfeld.[7] This mixture was then incubated at 37°C. for 36 hours. It was then extracted by shaking with ether, the ether extract was dried with anhydrous sodium sulfate and metallic sodium, and evaporated to dryness by vacuum distillation in a stream of nitrogen. The residue was taken up in 5 cc. of anhydrous chloroform. To 1 cc. of this solution was added 1 cc. of the antimony trichloride reagent. The appearance of a blue color is interpreted as being due to the presence of vitamin A.

Conclusions. 1. Carotenase is active in solutions of pH 7.4 and inactive in solutions on unfavorable acidity or alkalinity.

2. Carotenase is destroyed by cold.

3. In solutions of pH 7.4 carotenase maintains its activity for a considerable period of time if kept at 37°C.

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* The crystalline carotene was obtained from the Mead Johnson Laboratories.

[7] Fodor, A., and Schoenfeld, M., *Biochem. Z.*, 1931, **233**, 243.

6288

A New Hormone of the Anterior Pituitary.

OSCAR RIDDLE, ROBERT W. BATES AND SIMON W. DYKSHORN.

From the Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Riddle and Braucher[1] showed that the effective stimulus for the enlargement and functioning (formation of "crop-milk") of the crop-glands of pigeons is a substance derivable from the anterior hypophysis only; and the luteinizing substance derived from pregnant urine is not that substance. They were unable to decide "whether the principle activating the crop-gland is the growth, the sex maturity, or a third and now unknown anterior pituitary hormone." We have now shown (a) that the principle which evokes the crop-gland response is a third anterior pituitary hormone, and (b) have identified this same hormone, which we shall here call "Prolactin" as the hitherto undefined pituitary principle which is essential for lactation in mammals.

To prepare this hormone relatively free from the growth and gonad-stimulating principles frozen anterior pituitaries of beef or sheep were ground, defatted with acetone and alcohol and dried. This powder was extracted 3 times in aqueous medium at a pH of approximately 2.5. The acid extracts were precipitated isoelectrically. The isoelectric precipitate was redissolved and reprecipitated 3 times to free it of maturity hormone and then dried with acetone. About 10% of the original weight of dried powder is thus obtained in an acid-soluble isoelectric-insoluble form. The addition (to suspensions) of 0.2% cresol to complete the destruction of the growth principle does not markedly affect the "Prolactin".

Assays of several of our preparations, and some of the growth and gonad-stimulating extracts of others, have been made on immature common pigeons and ring doves of both sexes. Table I shows only a part of data from male ring doves. The crop-gland response is equally decisive in either species and sex; the gonad-stimulating response is more pronounced in males. Neither species has been adequately tested as to its suitability for the assay of the growth hormone; we therefore show here the absence of the "Prolactin" effect from growth hormone preparations of Drs. M. O. Lee and M. K. Schaffer, Boston, who kindly supplied very potent sam-

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[1] Riddle, O., and Braucher, P. F., *Am. J. Physiol.*, 1931, **97**, 617.

TABLE I.

Intramuscular injections of anterior pituitary preparations into male ring doves showing that growth and gonad-stimulating principles do not activate the crop-gland; "prolactin", practically free of the "maturity" principle, does activate the gland.

Preparation	cc. per day	Duration	Age of bird	Body wt.	Testes		Single crop gland
					Treated animal	Average control	
		days	mo.	gm.	mg.	mg.	mg.
Growth hormone* No. 36	.2	5	3.1	139	28.9	10.5	99
	.4	5	3.3	142	61.7	13.9	74
	.8	5	3.3	128	168.6	13.9	128
Growth hormone No. 31[1]	.6	5	3.1	135	35.3	10.5	73
Growth hormone[2] No. 31 + Prolan	.4	7	2.6	155	26.1	7.0	105
	.4	7	3.0	145	31.2	9.5	159
Prolan[3]	.2	5	3.0	143	6.9	9.5	84
	.2	7	2.9	150	10.1	8.6	120
Antuitrin (anterior lobe)	.3	9	2.7	132	67.0	7.6	96
	.3	9	2.8	144	118.3	8.5	67
"Prolactin"	.5[4]	6	2.9	129	8.8	8.6	740
	.5	6	2.8	167	8.9	8.5	1050
	.3	8	2.6	131	9.0	7.0	390
	.4	5	2.1	164	3.9	5.9	880
	.2	5	2.4	155	7.5	6.8	960
	.5[4]	4	2.6	148	7.5	7.0	985
	.5	4	2.3	123	7.4	6.3	925
	.5	4	2.1	144	8.3	5.9	765

* Lee and Schaffer; 0.4 cc. daily per 100 gm. rat gave maximum growth.

[1] Lee and Schaffer; 0.15 cc. daily per 100 gm. rat gave maximum growth.

[2] Each 0.2 cc. daily.

[3] Antuitrin S (Parke, Davis and Co.), from pregnant urine; 100 R. U. per cc.

[4] Represents 10 mg. of acid-soluble isoelectric-insoluble fraction.

ples assayed on hypophysectomized rats. Antuitrin (fresh) gives good gonad-stimulation in immature birds, but is devoid of the crop-gland stimulant. Prolan added to growth hormone was also without effect.

Male and female mature guinea pigs and mature female rabbits have responded to daily or twice daily subcutaneous injections of "Prolactin" by milk secretion—the males after preliminary treatment with theelin and progestin. The gonad-stimulating principle, freed of "Prolactin", fails in these tests; and the fresh growth principle is, by these and other tests, practically excluded. The secretion begins after 2-3 days in rabbits, 3-5 days in guinea pigs; the quantity is highly variable, and the term of secretion also variable. A resumption of milk secretion 5-7 days after cessation of treatment occurs in some cases.

6289

Secretion of Crop-Milk in the Castrate Male Pigeon.

OSCAR RIDDLE AND SIMON W. DYKSHORN.

From the Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Riddle and Braucher[1] showed that the cyclical extreme enlargement of two areas of the crop wall, and the resulting crop-milk formation—both well known to occur at or near the end of the brooding period in pigeons—are under control of a hormone of the anterior pituitary. Only anterior pituitary tissue or extracts were found capable of initiating these processes, but with such tissue or extracts these phenomena could be produced in birds of both sexes and at all ages. While unaware of the results of Riddle and Braucher a report was later published by Kaufman and Dabrowska[2] in which the conclusion is drawn that the hormone of the testis is necessary for the normal cyclical functioning of the crop-gland in the male pigeon.

Kaufman and Dabrowska castrated a male pigeon which had previously secured a female mate within a group of pigeons. After the male was castrated he ceased to copulate and express ardor, but nevertheless still maintained a special interest in his former mate. This female mate continued to lay fertile eggs—thus showing that another male (or other males) were copulating with her, and was wholly or in part stimulating the growth and ovulation of the eggs which she laid. It would therefore be a matter of much surprise if the castrate male *began* to incubate at the time this female laid eggs; for, even an unmutilated male pigeon who has been solely responsible for stimulating egg production in his mate is sometimes 2 to 5 days out of syncrony[3] with his mate in the impulse to begin to brood or sit; again, apparently normal males sometimes brood for a few days only and fail to complete the cycle. Indeed, it is of much interest that a castrate pigeon will incubate at all, and (though apparently unaware of this) Kaufman and Dabrowska have observed what seems to be the first recorded case. These authors, however, entirely fail to state whether this castrate *began* incubation as soon as eggs were laid by his mate or only 5 or 10 days thereafter; also whether the incubation was consistently and earnestly done; and whether perhaps he performed this function equally, but quite perfunctorily, *at all times* when eggs were placed in the nest of his mate. All these points have an important bearing on the significance of their finding that, when killed at the time its mate's young were hatched, the crop-glands of this (complete) castrate were entirely inactive. Unless this male had performed true incubation during the 13 or more days immediately preceding autopsy there would be no reason to expect anything other than inactive crop-glands. The fact that this castrate—operated when adult and probably after it had fed young—still showed the instinct to feed young is interesting but of uncertain meaning in relation to the point discussed here.

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[1] Riddle, O., and Braucher, P. F., *Am. J. Physiol.*, 1931, **97**, 617.

[2] Kaufman, L., and Dabrowska, W., *C. R. Assoc. d. Anat.*, Warsaw, 1931, Aug.

[3] Whitman, C. O., Behavior of Pigeons, Carnegie Inst. Publ. 257, 1919, **3**.

First Test. We have made 2 tests of the capacity of the crop-glands to enlarge and function in castrate male pigeons at the end of cycles of incubation which we knew were full and normal cycles: A normal mated pair which had produced and incubated earlier eggs was permitted to go through the copulations requisite for the production of a pair of eggs (laid April 24-26). The male was castrated on April 25—the date on which normally he would lightly begin incubation. Except for light sitting during the following 2 days, apparently because of sore wounds, he nested consistently. The 2 young were hatched on May 12. It was then proved (by removing the female) that the castrate could and did *feed* the young (May 12-13). On May 13—*i. e.,* 18 days after castration and beginning incubation—the castrate was killed for the necessary inspections. The castration was found to have been completely accomplished. A considerable part of the left, and a smaller part of the right, epididymis remained *in situ;* these were removed and sectioned. The 2 crop-glands were actively secreting crop-milk. The weight of the right gland was found to be 4.200 gm. Since this area when entirely inactive weighs approximately 0.200 gm. (in this race) it was 20 times its weight in the inactive state. The sectioning of such crop-glands is wholly superfluous; weights (or even appearance) are entirely decisive.

Second Test. Another (larger) male was castrated under conditions entirely similar to the case described above. This castrate also incubated consistently till both young were hatched 18 days later. The castrate fed these young at once, and was killed on this same day. The crop-glands were actively secreting, the weight of the right gland being 5.400 gm. Close examination of the former site of the testes showed no trace of gonad tissue on the left side; on the right side a small nodule (6.6 mg.) of regenerating testis tissue was found. Even at the date of autopsy the amount of this tissue was only about one-fourth of 1% of the amount of testis tissue removed at castration.

Injection Tests. Further to test the capacity of anterior pituitary extracts to activate the crop-glands in castrate male doves and pigeons 2 additional castrates were treated as follows: A male which had been castrated (completely) 6 months earlier was given 4 daily injections of an alkaline extract of the anterior lobes of cattle. Ninety-six hours after the first injection the right crop-gland, though not actively secreting, had attained almost or quite to the maximum (1.900 gm.) to be expected from the dosage, and would doubtless have begun secretion within the next 24 hours. A second, and wholly similar test was made on a ring dove, also castrated 6 months before the administration of the pituitary extract. Here the crop-glands were actively secreting and the right gland (normal inactive weight=about 0.100 gm.) weighed 0.930 gm. Here again we obtained the maximum enlargement to be expected from dosage during a 4-day period.

Summary. The castration of male pigeons at the beginning of an incubation cycle, to insure true and consistent incubation, does not even slightly interfere with the enlargement and complete functioning of the crop-glands at the end of the incubation cycle 18 days later.

Castration of adult male pigeons and doves 6 months prior to the administration of potent extracts of the anterior pituitary does not lessen or otherwise modify the capacity of these extracts to activate the crop-glands of these birds.

There is no valid evidence that the testes play any direct part in the activation of the crop-glands. The testes do, of course, induce or influence the copulations which normally lead to the males' impulse to incubate; and a completed term of incubation apparently induces the anterior pituitary to release the specific hormone which in turn activates the crop-glands. The presence of this activator in the blood is as effective in the absence as in the presence of the testes.

6290

Relative Growth Rates in Large and Small Races of Pigeons.

OSCAR RIDDLE, DONALD R. CHARLES AND GEORGE E. CAUTHEN.

From the Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, L. I.

Castle and Gregory[1,2] showed that cell multiplication is more rapid in the eggs of a large race of rabbits than in one of small adult size, although the absolute size of the eggs before cleavage and the rate of differentiation during prenatal growth were not different in the 2 cases. Here the adults of the large race were fully 3 times as large as those of the small race; at birth, however, individuals of the large race were only twice the size of newborn of the small race. Painter[3] had earlier obtained evidence that 12-day embryos of this same large race of rabbits contained more cells than did embryos of the small race when studied at the same age. Finally, Robb[4] found that the percentage growth-rate from birth to puberty is the same in these 2 races of rabbits, but after puberty this rate is more depressed in the small race. He failed to find evidence that pituitary difference is at any stage the basis of the growth difference.

In the present study we wished to learn from large and small races of common pigeons the extent to which differential growth is demonstrable by size at hatching, and the extent and time-relations of differential growth rates in the post-hatching period. This limitation of our problem relieves us here of the citation of a large body of literature. While this study was in progress Byerly[5] published rather extensive data concerning the growth of embryos from a larger (2.77 kilos) and a somewhat smaller (2.01 kilos) race of fowls. From the 10th day onward it was found that the embryos of the heavier race were slightly heavier than those of the lighter race; but in eggs of the same weight from the 2 breeds the size difference tends to disappear toward hatching time, probably due to the equivalence of a strictly limited food supply. A relation between size of egg and ultimate size of the contained embryo was indeed earlier well known in fowls, and in this laboratory it has long

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[1] Castle, W. E., and Gregory, P. W., *J. Morph. and Physiol.*, 1929, **48**, 81.

[2] Gregory, P. W., and Castle, W. E., *J. Exp. Zool.*, 1931, **59**, 199.

[3] Painter, T. S., *J. Exp. Zool.*, 1928, **50**, 441.

[4] Robb, R. C., *Brit. J. Exp. Biol.*, 1929, **6**, 293.

[5] Byerly, T. C., *J. Morph. and Physiol.*, 1930, **50**, 341.

been recognized to hold for pigeons. Kaufman[6] has published a series of papers dealing with body and organ growth in a race (unnamed) of pigeons, and in one of these studies[7] has compared the rate of growth in chick and pigeon embryos. Data showing the exceptionally high rate of growth of ring doves and pigeons have been reported by Riddle[8] and by Lienhart.[9]

Four well-known and long-established races were chosen for study. We may here consider the weight of all these birds at 105 days (after beginning development; 87 days after hatching) as representing the mean adult weight of the 2 sexes. It is true that these birds all increase very slightly in weight—particularly during autumn and winter—after 105 days; but by using these fairly accurate figures we avoid practically all error due to seasonal change, since the present weights were obtained from March to August, inclusive. The adult weights, thus defined (and confirmed by the weights of numerous adults), are: For Tipplers, 278 gm.; Magpies, 296 gm.; Homers, 398 gm.; Runts, 565 gm. The largest race (Runts) is thus twice the size of the smallest (Tipplers).

Weights were obtained on the newly hatched young and (on some or many of these young) at 3-day intervals thereafter. The data thus obtained for the 4 races are presented in the form of smoothed curves. Eggs of pigeons are incubated unequally and they hatch between the 17th and the 19th day, usually on the 18th day. In any event their weight was obtained at hatching and at 3-day intervals thereafter, and the age at hatching is uniformly considered 18 days; at 3 days after hatching the bird is, by our method of reckoning, 21 days old. The weights as given are true weights of the body after deducting (when necessary) the weight of the contents of the crop.

There are several sources of error in obtaining the true growth-curve of any race of pigeons. When there are 2 young in a nest they each grow less rapidly than if only one were present. When a squab has attained a weight of 100 gm., and remains healthy, it will probably attain to four-fifths of its adult weight within the next 7, 15, or 25 days—depending normally upon the amount of food it receives from its regurgitating parents. An example of this decisive influence of extraordinary food conditions was shown by a bird of mixed blood whose adult weight is, and was expected to be, about

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[6] Kaufman, L., *Biol. Generalis*, 1927, **3**, 105.

[7] Kaufman, L., *Mem. Inst. Nat. Polonais a Pulawy*, 1929.

[8] Riddle, O., *Am. J. Physiol.*, 1928, **86**, 248.

[9] Lienhart, R., *C. R. Soc. Biol.*, 1931, **107**, 47.

530 gm. Fed as the sole occupant of a nest by extraordinary parents this bird weighed 346 gm. on the *twelfth* day after hatching. In view of this great source of variability in animals measured during the 24-day to 50-day stages of growth a large number of weights is here of less significance than a much smaller number obtained from healthy birds under essentially standard conditions. During the collection of these data many of the young taken for measurement became infected with intestinal *Capillaria,* or developed other disease. We have either discarded such data entirely, or retained only such weights as were obviously obtained before the onset of disease. It thus happens that we have recorded more weights for early growth-stages than for later ones. The data presented include 1098 individual weights; of these 443 are for Tipplers, 177 for Magpies, 336 for Homers, and 142 for Runts. The decrease in weights soon following the 50-day stage reflects the fact that the young birds were removed from their parents, and assumed wholly self-feeding habits, at this period.

There is one source of apparent error—really involving a peculiar or abnormal condition of egg-size in one part of our material—that could not be avoided by us. Our stock of Runts was showing various ill effects of inbreeding before these studies were started. It happens, therefore, that our measurements on the young of this race have been limited to valid measurements on only 3 to 6 individuals, except for 19 weights of embryos at hatching. But it is precisely these weights at hatching that contain unavoidable error, since the inbreeding of this race has led to, or has been accompanied by, a marked reduction in egg size—and therefore of embryo size—during the past few years. Before intensive inbreeding the eggs of this race had an average weight (110 eggs) of 22.5 gm.; but during the past 2 years, which is the period covered by this study this average (170 eggs) has decreased to 19.9 gm. The embryo weights at hatching have almost or quite certainly undergone a like reduction. Our data show that this handicap (as compared with the large Homers) on hatching weight persists at 3 and 6 days after hatching; but in all of the following 27 periods the young Runts are larger than those of the Homers of corresponding age. This abnormality of the weights of Runt embryos at hatching, with rapid subsequent recovery of normal size, well shows therefore that an environmental limitation (diminished egg size) during this early period of embryonic growth does not prevent the expression of the genetic or other growth factors in post-natal growth.

At hatching the average weights of the embryos of the 4 races were as follows: Tipplers (19), 11.3 gm.; Magpies (13), 11.5 gm.; Homers (16), 14.7 gm.; Runts (19), 14.0 gm. Since the adult weights of these races were 278, 296, 398 and 565 gm., respectively, it is clear that a part—though only a part—of the racial size difference is expressed by the weights at hatching. For the 2 smaller races the difference at the 2 periods is slight—this being about 2% at hatching and 6% in adults; for Tippler and Homer, we find 30% at hatching and 43% in the adults; for Tippler and Runt (abnormal), 24% at hatching and 103% in adults. For Magpie and Homer these values are 28% and 34%; for Magpie and Runt (abnormal embryo size), 22% and 91%. For the two large races, Homer and Runt, the embryos of the former are actually 5% larger than the (abnormally small) embryos of the Runt, though the adult Runts exceed the Homers by 42%. Thus, in all cases the racial size difference of pigeons is only partially expressed in the size of the embryos at hatching.

A large share of the ultimate size difference in these races is attained through differential rate of growth increment after hatching and most of this increment occurs during the first 18 days after hatching. During this 18-day period (18th to 36th day) the average daily percentage increment is 23.3 in Tipplers, 24.6 in Magpies, 25.7 in Homers, and 27.5 in Runts. *These values stand in the same relative order as does adult body weight in the 4 races concerned.* Instantaneous relative growth-rates calculated from fitted curves (for Tipplers, Homers and Runts) demonstrate the significance of these percentage increments. This differential rate of growth in the earliest stages after hatching provides an interesting

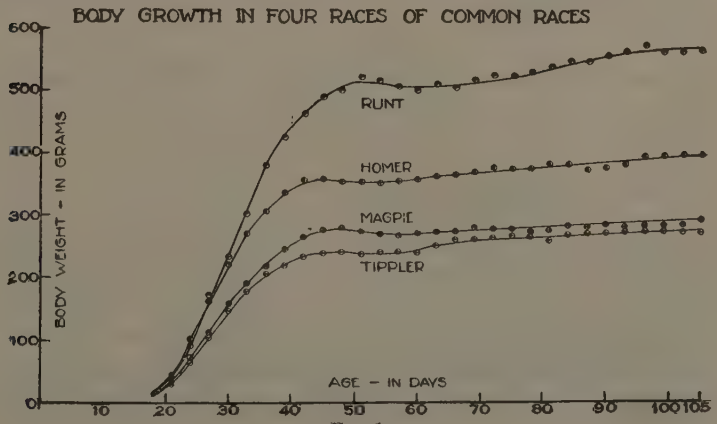

Fig. 1.

variation from the conditions described by Robb in the large and small races of rabbits otherwise studied by Castle and Gregory. There the rate of post-natal growth was identical in the 2 races until after puberty; in pigeons the total size difference is attained before puberty. This difference, however, is in no way disconsonant with the basic contribution of Castle and Gregory.

Summary. Data on relative growth-rates have been obtained for 2 small and 2 large races of common pigeons from the time of hatching to a period, 87 days later, at which their adult body weight is practically attained.

The weights of embryos of these 4 races at hatching demonstrate that racial size difference is only partially and very imperfectly expressed by size differences attained during embryonic life. A large part of racial size difference in pigeons is rapidly attained early in the post-hatching period by differential growth-rate, and practically all of adult size difference is attained before puberty.

These are marked variations from the time relations of differential growth in the large and small races of rabbits studied by Castle and Gregory, but such variations offer nothing adverse to their main conclusion. We consider it an open question, to be investigated within each species or group, whether and to what extent endocrine (pituitary) difference influences racial differences of growth-rate at a particular developmental stage; but wholly probable that such endocrine difference is an effective factor in the growth of higher animals at one or another stage.

6291

Glycosuria in Rabbits Following Injections of Saline Extract of Anterior Pituitary.

EMIL J. BAUMANN AND DAVID MARINE.

From the Laboratory Division, Montefiore Hospital, New York.

A relation of the anterior pituitary to carbohydrate metabolism has often been suspected on the basis of the frequent occurrence of hyperglycemia and glycosuria in acromegaly, and of anatomical changes in the anterior pituitary in diabetes mellitus, but such a connection was not indicated experimentally until Houssay and Biasotti[1] showed that pancreatic diabetes could be prevented in toads by removal of the anterior pituitary. Subcutaneous implantation of anterior pituitary in such depancreatized toads re-established the diabetes. They were unable to show a similar relation in mammals either by gland implantation or by injections of anterior pituitary extracts, although depancreatized and hypophysectomized dogs survived longer and showed milder diabetes than did depancreatized controls[2]. Evans, Meyer, Simpson, and Reichert[3] reported observations in which they produced glycosuria and polyuria in 2 of 4 dogs by daily injections of the growth hormone fraction of anterior pituitary extracts for 8 or 9 months.

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[1] Houssay, B.-A., and Biasotti, A., *Comp. rend. de la Soc. biol.*, 1930, **104**, 407; 1930, **105**, 121.

We have made somewhat similar observations in rabbits. Hyperglycemia, polyuria, glycosuria, and lipemia developed after injecting a saline extract of ox pituitary daily for 6 or more days, in some instances of surprisingly great intensity. It occurred in each of the 4 rabbits used, though varying in degree.

The extract was prepared twice weekly from fresh chilled glands, according to the method of Schockaert[4] except for the measures used in sterilization. It produced typical reactions among which were rapid hypertrophy of the mammary glands with lactation, luteinization of the ovaries, hypertrophy of the thyroid and suprarenal cortex.

The rabbits were kept on our stock diet of alfalfa hay and oats. Two were injected with 2 cc. and 2 with 4 cc. daily, equivalent to one-half and one anterior pituitary respectively. The data are summarized in the following table:

Collection	2 cc. ext. daily				4 cc. ext. daily			
Period	R 1039		R 1073		R 1041		R 1097	
	Glucose	Amt. per day	Glucose	Amt. per day	Glucose	Amt. per day	Glucose	Amt. per day
days	%	gm.	%	gm.	%	gm.	%	gm.
6-7	5.0	8.1	0.5	0.4	0.4	0.5	0.4	0.4
8	5.1	10.2	0.6	0.6	6.9	16.7	0.6	0.7
9-10	4.8	6.6	1.6	1.1	10.4	15.7	1.4	1.4
11	5.3	10.2	1.0	1.1	14.5	34.9	3.9	5.8
12-13	5.1	8.3	1.3	1.2	9.0	18.6	5.6	7.6
14-15	0.4	0.7	0.6	0.5	9.1	15.7	1.2	1.3
16-17*		trace?		trace?	5.2	7.8	0.4	0.4
18-19		neg.		neg.		neg.		neg.

*Last injection on 17th day.

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[2] Houssay, B.-A., and Biasotti, A., *Pflüger's Arch.*, 1931, **227**, 664.

[3] Evans, H. M., Meyer, K., Simpson, M. E., and Reichert, F. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 857.

[4] Schockaert, J. A., *Anat. Record*, 1931, **50**, 381.

Analyses were made on the urine in the collection bottles combined with the washings from the metabolism cages. The values in the table are not strictly 24 hour ones, but the total amount for the entire period is complete. Periods of one or 2 days were used as indicated; the amount excreted is calculated per day. The percent of sugar is lower than that of the urine as excreted due to dilution of the urine with cage washings. In one of the rabbits there was a very high percentage of sugar in the urine, as secreted, amounting to 18% on the 11th day.

The exact date of the onset of glycosuria unfortunately is not known. We did not suspect the presence of sugar in the urine until the sixth day when our attention was drawn to the large volume of urine excreted and the intense heat generated on the addition of chromic acid. In rabbit 1039 glycosuria was marked on this day, while in the other rabbits there was only 0.4-0.5% of sugar in the urine. In these 3 it is probable that glycosuria did not appear before the fifth day, while in rabbit 1039 the onset may have occurred on the third day. The intensity of the glycosuria was somewhat independent of the amount of extract injected, probably due to variations in susceptibility.

After 11 daily injections the morning blood sugar levels on rabbits 1039, 1041, 1073, and 1097 were 475, 348, 240, and 122 mg.% respectively (by the method of Folin[5]). The sera of these bloods were very milky. Ten days after the last injection the blood sugars of 1073 and 1097 were 92 and 93 mg.% respectively and the sera were clear. In this connection it should be remembered that the blood lipids of rabbits are quite low.

Since saline extracts are unstable they may have deteriorated during the 4 days they were kept. The extract used for the injections on the 14-17th days may have been inactive because the glycosuria decreased beginning on the 14th day. The possibility that the animals became resistant to the effects of pituitary extract must be considered. Injections were discontinued on the 17th day; 2 or 3 days after injections were stopped, glycosuria disappeared completely.

That the reducing substance in the urine was glucose was shown by making the phenylosazone. Its crystalline form and melting point corresponded to that of glucososazone. Clarified urine was strongly dextrorotatory.

Infection was not a factor in these findings.

In spite of large losses of sugar in 2 instances (50 gm. and 120 gm. in 6 days), there were no significant changes in body weight. The pH of the urine varied between 8.5 and 9.5, usual for rabbits on our stock diet.

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[5] Folin, O., *J. Biol. Chem.*, 1929, **82**, 83.

We have also tested in 4 rabbits the effect of "phyone"† which according to Van Dyke[6] contains the pituitary growth hormone. Rabbit 1060, male, after injections of 1 cc. of phyone for 3 days had 0.5% sugar in its urine. The daily injections were continued and on the 4th day 5.6 gm. of sugar was excreted and on the 5th and 6th days 8.9 and 10.4 gm. respectively. Injections were stopped and the sugar output dropped to 4.8 gm., then to 2.6 gm., and 3 days after cessation of phyone administration no sugar was excreted. Five days later the blood sugar was 106 mg.%. Rabbit 1068 after injections of 1 cc. for 5 days and 2 cc. for 10 days showed no glycosuria. After the last injection the morning blood sugar was 100 mg.%. Rabbit 1063 had a trace of sugar in its urine after a single injection of 1 cc. of phyone but this did not increase although it received 10 cc. during the next 8 days. The blood sugar was 129 mg.%. Rabbit 1071 received 11 cc. of phyone during a period of 8 days. On 2 occasions a questionable test for sugar was obtained in the urine and on the last day its morning blood sugar was 121 mg.%.

Our results with phyone are similar to those of Evans, Meyer, Simpson and Reichert in that only one of the four rabbits developed a severe glycosuria. Since a much more regular and definite response was obtained with saline extracts, one is led to infer either that a material loss in activity must have occurred in the preparation of the growth hormone fraction (equivalent amounts in terms of anterior pituitary of both phyone and saline extract were used), or that the hyperglycemia and glycosuria are caused by something distinct from the growth hormone.

Respiratory quotients have been determined in 2 rabbits rendered hyperglycemic and glycosuric by the Schockaert saline emulsion but the results so far are doubtful and quotients varying between 78 and 85 have been obtained, indicating that the animals were utilizing sugar.

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†Kindly supplied to us by Wilson and Co. through the courtesy of Dr. David Klein.

[6] Van Dyke, H. B., and Wallen-Lawrence, Z., *J. Pharm. and Exp. Therap.*, 1930, **40**, 413.

6292

Uterine Bleeding of the Interval Type in Macacus Monkey During Injections of Extracts of Pregnancy Urine.*

E. T. ENGLE

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Uterine bleeding in the spayed or immature macacus monkey is known to occur from an interval type endometrium about 6 days after cessation of an adequate treatment with oestrin (Theelin, Allen[1], Maddux[2];) (Amniotin, Morrell *et al.*[3]). These observations have been repeatedly confirmed in this laboratory. Uterine bleeding in the adult also occurs after bilateral ovariectomy (Allen; Van Wagenen and Aberle[4]) or occasionally after unilateral ovariectomy (VanWagenen and Aberle). (Uterine bleeding indicates a hemorrhage of uterine origin macroscopically visible on the external genitalia of the monkey, but which may not be homologous with menstruation). Saiki[5] has shown that uterine bleeding may be induced in immature macaques 4 to 9 days after the cessation of 8-17 days treatment with an extract of anterior lobe (P. D. & Co.). Our work confirms these results. However, if water soluble extract of pregnancy urine or the "luteinizing hormone" (P. D. & Co.) be administered in adequate doses for from 19 to 22 days, in 2.8 to 4 kg. animals, bleeding occurs *during the course of treatment.* If the injections are continued, bleeding stops in 4 to 6 days, after an apparently normal flow.

Injections of extracts of pregnancy urine have failed to cause reddening of the sexual skin, or induce the turgose, pachydermatous condition of the circumgenital and anal area which is obtained by injections of oestrin or is seen after ovarian stimulation by means of water soluble fraction of pyridine extract (Hisaw, Fevold and Leonard[6]). No follicular cysts, nor any determinable growth of the ovarian follicle occurs after such injections. Modification of the structure of large atretic follicles may be obtained after continued intravenous injection of pregnancy urine, but no structures which are clearly corpora lutea have as yet been obtained with this substance, although luteinization in the rat is a pronounced result after prolonged subcutaneous intraperitoneal or intravascular injections.

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*Aided by a grant from the National Research Council, Committee for Research in Problems of Sex.

[1] Allen, E., *Cont. to Embryol., Carnegie Inst.*, 1927, 19.

[2] Maddux, W. P., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 873.

[3] Morell, J. A., *et al., Endocrinol.*, 1930, **14**, 174.

[4] VanWagenen, G., and Aberle, S. B. D., *Am. J. Physiol.*, **99**, 271.

[5] Saiki, S., *Am. J. Physiol.*, **100**, 8.

[6] Hisaw, F. L., Fevold, H. L., and Leonard, S. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 204.

In the female monkey the only well recognized phenomena which occurs after treatment of normal intact animals with pregnancy urine extracts is this uterine bleeding. The ovaries, although they show no morphological change as yet recognizable, are essential in this mechanism for bleeding, as no results were obtained in an ovariectomized animal even after 30 days of treatment. In no case has the endometrium shown the degree of development which is obtained by means of oestrin, or after stimulation with the water soluble pyridine extract of anterior pituitary. Bleeding in every case studied has occurred from an interval type endometrium, with poor glandular development and with relatively little tissue destruction.

6293

Prevention of Experimental Uterine Bleeding in Macacus Monkey by Corpus Luteum Extract (Progestin).*

P. E. SMITH AND E. T. ENGLE.

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Since uterine bleeding was shown to follow cessation of follicular treatment, the many observations on experimental menstruation in the monkey have not given adequate consideration to the rôle of the corpus luteum. Experiments were designed to study this question. Adult or subadult animals, all beyond the first menstruation (3700 to 5150 gm. B.W.) were injected with the follicle stimulating water soluble fraction of sheep pituitaries (Pyridine method of Fevold, Hisaw and Leonard) for 10 to 14 days. Coincident with the last 4 of these injections oestrin (Theelin) was also given in large doses (100 R.U. per diem)**. The uterus at the end of the treatment was large, firm and of a deep red color. It is known that the animal will bleed within 6 days of the cessation of this treatment. Bilateral ovariectomy was also performed, which alone will be followed by uterine bleeding. On this day of ovariectomy, treatment was begun with Progestin (method of Corner and Allen), giving generally 70 gm. equivalent of fresh sow corpora daily***. *Bleeding has never occurred during the course of treatment with progestin,* and has been prevented, in various animals for from 10 to 28 days after the double stimulus to bleeding afforded by bilateral ovariectomy and cessation of oestrin treatment. The preparation has one R.U. of oestrin per 19 gm. equivalent which is quite inadequate to prevent uterine bleeding.

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*Aided by a grant from the National Research Council, Committee for Research in Problems of Sex.

**The Theelin and dried sheep pituitary glands were kindly supplied by Parke Davis & Co., through the courtesy of Dr. O. P. Kamm.

A typical animal (My89, wt. 4.4 K.) was given 15 days of aqueous solution of anterior pituitary (dry gland equivalent of 17 gm.). During the last 4 days of this period 100 R.U. oestrin (Theelin, P. D. & Co.) was administered daily. On operation the ovaries were large (1.172 gm.) and contained numerous cystic follicles, no corpora. Both ovaries were removed. The uterus was firm and measured 18 x 14.7 mm. Progestin (70 gm. fresh gland equivalent daily) was continued for 28 days. Bleeding began on the sixth day following the last injection of progestin.

A typical progestational endometrium is obtained by the 10th day of treatment, similar to that previously obtained by Hisaw, Meyer, and Fevold[1]. The bleeding which follows the breakdown of the progestational endometrium is more profuse and of a different quality than interval type bleeding.

In the monkey and human, uterine bleeding occurs in the absence of ovulation and of corpora lutea. The impression obtained over an extended period of observation with experimental induction of uterine bleeding following cessation of injections of oestrin (theelin, amniotin) and a luteinizing preparation of the anterior pituitary (P. D. & Co.) or during the course of injections of pregnancy urine extract, is that the type and quality of bleeding is not the same as after cessation of progestin treatment. Description of the tissues and of blood extravasation from the endometrium will be published later. Provisionally, we are applying the term menstrual bleeding only to that type of uterine hemorrhage which follows the degeneration and sloughing of a prepared progestational endometrium.

***The fresh corpora lutea were supplied by the Wilson Laboratories, through the courtesy of Dr. David Klein.

[1] Hisaw, F. L., Meyer, K. K., and Fevold, H. L., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 400.

Uterine bleeding from the interval endometrium with proportionately less tissue loss, and the passing of red cells through the epithelium by diapedesis may not be a homologous process.

6294

The Question of Synthesis of the Antirachitic Vitamin by the Cod.

ALFRED F. HESS, CHARLES E. BILLS, MILDRED WEINSTOCK AND MIRIAM IMBODEN.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, and The Research Laboratory, Mead Johnson & Co., Evansville, Ind.

Ever since the recognition of the antirachitic factor, the question has been raised as to whether cod fish synthesize this factor or whether the marked potency of their liver oil is due to antirachitic vitamin taken in with the food. Investigations have been few and inconclusive. Wejdling[1] has drawn attention to the fact that diatoms, which may form a part of the diet of the cod, contain the antirachitic vitamin. On the other hand, Bills[2] has demonstrated that caplin, which are consumed in enormous quantity by the Newfoundland cod, are not rich in the D factor, and suggested that some of the vitamin may be formed by synthesis within the fish.

Some years ago we carried out experiments designed to ascertain whether the livers of cod fish which were fed large amounts of non-activated ergosterol by mouth contained exceptional amounts of the antirachitic factor. Cod fish were given, by means of a pipette, 5 feedings of 10 mg. each of ergosterol suspended in cottonseed oil. The fish were kept in tanks in New York Bay and feedings were given at 2-day intervals, the last one being given about 36 hours before the fish were killed. The result may be summarized by the statement that the livers of the fish which received the ergosterol were found, on biological assay, to contain the antirachitic vitamin in no greater amount than the control fish which were kept under similar conditions, but which had not been given ergosterol. Two series of experiments were carried out with the same result.

Last December this subject was approached from a somewhat different point of view. Instead of giving ergosterol by mouth, it was injected intramuscularly. Three injections were given of 1 mg. dissolved in 5 cc. of corn oil; there was an interval of 2 days between each injection and 2 days elapsed before killing the fish. In March of this year a second experiment of this kind was carried out, as it was thought that it would be of advantage to make tests at 2 different seasons of the year for the oil in the liver of the cod undergoes marked seasonal variations. These 2 tests comprised 14 fish, 6 of which were injected and 8 served as controls. The first lot varied in weight from 4 to 4½ pounds and the later series from 2½ to 9½ pounds. The group contained about an equal number of male and female fish. Among the females in the March group, some were found to have spawned and some contained new roe. The males were spent.

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[1] Wejdling, K., *Acta paed.*, 1928, **7**, Suppl. II, 259.

[2] Bills, C. E., *J. Biol. Chem.*, 1927, **72**, 751.

These experiments gave no indication that the cod is able to activate ergosterol, in other words to elaborate antirachitic vitamin from ordinary ergosterol. The potency of the livers, in the group which was inoculated, ranged between 90 and 280 international units and in the non-inoculated group between 90 and 250 units. It may be added that the average cod liver oil used for therapeutic purposes contains about 80 international units per gm. These experiments furnish no evidence that ergosterol, whether given by mouth or intramuscularly, is utilized by the cod in the elaboration of vitamin D, but do not preclude the ability of these fish to synthesize this vitamin under different conditions.

6295

Effect of Methylene Blue on CN and CO Poisoning.*

M. M. BROOKS.

From the Department of Zoölogy, University of California.

According to Warburg,[1, 2, 3], both KCN and CO inhibit aerobic respiration but not glycolysis. When aerobic respiration is inhibited, it becomes possible to show that certain redox indicators, such as methylene blue, accelerate aerobic glycolysis as indicated by increased oxygen consumption[4, 5].

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*Aided by grants from the Cancer Fund established at the University of California Medical School by a friend.

[1] Warburg, O., *Z. physiol. Chem.*, 1910, **66**, 305.

[2] Warburg, O., *Biochem. Z.*, 1926, **177**, 471.

[3] Warburg, O., *Biochem. Z.*, 1923, **142**, 518.

[4] Barron, E. S. G., *J. Biol. Chem.*, 1929, **81**, 445.

[5] Barron, E. S. G., and Harrop, G. A., *J. Biol. Chem.*, 1928, **79**, 65.

It was thought that increase in oxygen consumption thus produced by redox indicators might be used to combat the effects of HCN or CO on mammals. Previous workers have confined their attention to the effects of methylene blue used in conjunction with cyanide. Among other things it was found empirically that methylene blue favored the recovery of animals poisoned by cyanide[6, 7]. Since CO has long been known to resemble CN in its effects on respiration it was thought of interest to see whether methylene blue also favored recovery in animals subjected to CO.

Experiments were carried out using rats which were placed one at a time in a chamber containing HCN gas or CO (about 1%). The time to unconsciousness was noted. As soon as an animal became unconscious it was removed from the chamber and given an intraperitoneal injection of methylene blue. The time required for recovery was determined using as a criterion of recovery the ability to run straight forward. Eighty rats were used; 40 controls and 40 dye-treated.

It was found that the use of methylene blue shortened the time required for recovery from HCN poisoning to about ⅓, and that from CO to about ½ of that for untreated animals.

These results suggest that there is an activation of oxygen by methylene blue which manifests itself when aerobic oxidation is interfered with.

It is suggested that methylene blue injections could be used advantageously in cases of HCN or CO poisoning.

6296

Utilization and Excretion of the Female Sex Hormone.

ROBERT T. FRANK, MORRIS A. GOLDBERGER AND FRANK SPIELMAN.

From the Gynecological Service and the Laboratories, Mount Sinai Hospital, New York City.

Of importance are answers to the question of (1) what happens to the female sex hormone after injection, (2) how is it excreted, (3) can the excretion be stimulated?

1. *What happens to the female sex hormone after injection?* Half hourly blood samples taken from isolated adult rabbits in

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[6] Sahlin, Bo, *Skand. Arch. Physiol.*, 1926, **47**, 284.

[7] Eddy, N. B., *J. Pharm. and Exp. Ther.*, 1931, **41**, 449.

whom 2000 M. U. of female sex hormone had been injected intravenously in one dose, showed that only 1 M. U. could be recovered from 4 cc. of serum during the first hour, and none thereafter. After subcutaneous injection, 5 cc. showed this reaction during the first hour. Twenty-four hours later the blood of a rabbit injected with 3000 M. U. and killed 24 hours later by bleeding, showed less than 1 M. U. in 40 cc. of blood, one-half of the total blood volume. From another rabbit given 3000 M. U. and killed 24 hours later, extracts made from the total skeletal musculature, the lungs, brain and liver were assayed. With the exception of the liver, the total extract of the organ proved negative. The extract corresponding to one-half the total liver contained but 1 M. U.

This series of experiments shows that large amounts of female sex hormone given intravenously or subcutaneously in the rabbit, rapidly disappear from the circulation and cannot be recovered from the internal organs 24 hours after injection. These results do not show whether the hormone is destroyed or so changed within the body fluids or tissues as to be no longer demonstrable by the Allen and Doisy reaction.

2. *How is the female sex hormone excreted?* Allen[1] found that female sex hormone was excreted almost quantitatively when injected into monkeys. We injected 2 African green monkeys, a male and female, each with 1500 M. U. of the hormone subcutaneously. .Careful titer of the total urine for the next succeeding 2 days showed an excretion of approximately one-fifth of the amount of hormone injected.

An assay of the total amount of female sex hormone excreted in the urine of a normal fertile, normally menstruating woman, over one period was compared with the succeeding month during which time the individual received 5000 M. U. subcutaneously on the 9th, 15th and 26th day of the cycle. The differences in excretion of the entire amount during the month, as well as a comparison of the day before, during and after the injection, showed no appreciable changes.

Based on the observation of a large number of normal women, it is noted that although the blood samples during the first 3 weeks of the cycle show no variation, an increased excretion of female sex hormone in the urine occurs between the 9th and 11th day and again from the 24th to the 28th day. This might be interpreted as a short transitory flooding of the circulation with large quantities of female sex hormone suddenly elaborated by the follicle and corpus luteum. No evidence of this change in the blood has been found in a study of some 2000 patients. Another explanation is a possible variability in kidney permeability at different periods of the cycle. Such evidence as we have collected in substantiation of this hypothesis has been published previously.[2]

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[1] Allen, E., personal communication.

3. *Can the kidney permeability be influenced or excretion stimulated?* The experiments of Smith and Smith[3] lead them to believe that progestin stimulates the excretion of the female sex hormone.

Repetition of their experiments, using Antuitrin (Parke, Davis & Co.) as well as progestin supplied through the kindness of Dr. George Van S. Smith, gave negative results.

In our series, both the urine and feces were extracted and titered. Two normal isolated rabbits and one spayed female were used for the Antuitrin experiments, 900 M. U. of female sex hormone being injected. Two castrated adult females were used in the progestin experiments, the dose of female sex hormone being 1800 M. U. These experiments failed to confirm the results obtained by Smith and Smith. These results might indicate a rapid destruction or fixation of the female sex hormone in some non-assayable form but other interpretations cannot be excluded.

6297

Effect of Digitalis and Rest on Pulmonary and Peripheral Circulation in Patients with Circulatory Failure Caused by Heart Disease.

GEORGE P. ROBB AND SOMA WEISS.

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard) of the Boston City Hospital, and the Department of Medicine, Harvard Medical School.

This study was undertaken to shed further light on the mechanism of circulatory failure caused by heart disease and on the circulatory changes that follow administration of large therapeutic doses of digitalis, and rest. Weiss and Ellis[1] observed the effect of digitalis on the volume and velocity of blood flow and other aspects of the circulation after repeated control observations in patients with rheumatic heart disease and with compensated circulation at rest. In this study such repeated control observations on the aspects of the circulation measured, because of the clinical condition of the patients and of the methods used, were not feasible. A definite separation of the changes due to digitalis effect and rest between determinations, therefore, cannot be made, but the arrangement of the observations was similar to those used in the treatment of cardiac patients. To ascertain a possible correlation between the pulmonary circulation and ventilatory function, in some of the cases measurements of different components of the lung volume were also performed simultaneously with the circulatory observations. The following aspects of the circulation were studied simultaneously before and after digitalis: (1) electrocardiogram; (2) the degree of orthopnea; (3) arterial blood pressure; (4) venous pressure with the method of Moritz and Tabora[2]; (5) cardiac minute volume output with the method of Moore, Kinsman, Hamilton, and Spurling[3]; (6) the peripheral and pulmonary velocity of the blood flow according to the method of Weiss and Robb[4]; (7) blood volume according to the method of Keith, Rowntree and Geraghty[5]; (8) lung volume with the method of Van Slyke and Binger[6]; (9) oxygen and carbon dioxide content of the blood samples obtained from the femoral artery and vein.

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[2] Frank, R. T., and Goldberger, M. A., Proc. 2nd Int. Cong. for Sex Res., 1930, 378.

[3] Smith, G. Van S., and Smith, O. W., *Am. J. Physiol.*, 1931, **98**, 578.

[1] Weiss, Soma, and Ellis, L. B., *J. Clin. Invest.*, 1930, **8**, 435.

The patients were given powder of digitalis corresponding to 2 gr. or more per 10 lb. of body weight within 24 hours. A maintenance dose of 2 to 3 gr. was given daily thereafter. All 12 patients exhibited clinical evidence of circulatory failure at rest, but according to certain clinical manifestations and laboratory findings they were divided in 3 groups.

Group A comprised 4 patients with dyspnea and orthopnea and regular cardiac rhythm. As judged from the normal peripheral venous blood flow and the normal venous pressure, the normal oxygen difference between the femoral artery and vein and the absence of peripheral edema or enlargement of the liver, no obvious congestion or circulatory disturbance existed in the peripheral circulation. The dyspnea, orthopnea, rales over the bases of the lungs prolonged mean pulmonary velocity of the blood flow, low vital capacity, decreased ratio between the vital capacity and the residual air space of the lungs, indicate a disturbance of the pulmonary circulation as well as of the physiological state of the alveolar function.

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[2] Moritz, F., and Tabora, D. V., *Deutsch. Arch. f. klin. Med.*, 1910, **98**, 475.

[3] Moore, J. W., Kinsman, J. M., Hamilton, W. F., and Spurling, R. G., *Am. J. Physiol.*, 1929, **89**, 331.

[4] Weiss, Soma, and Robb, G. P., unpublished study.

[5] Keith, N. M., Rowntree, W. G., and Geraghty, G. T., *Arch. Int. Med.*, 1915, **16**, 547.

[6] Van Slyke, D. D., and Binger, C. C., *J. Exp. Med.*, 1923, **37**, 457.

Group B comprised 4 cases with regular cardiac rhythm, which, however, from the clinical observations and the above-mentioned measurements in addition to the pulmonary congestion, exhibited also congestion of the peripheral circulation.

Group C included 4 cases with auricular fibrillation, who similarly to the cases of Group B in addition to pulmonary congestion, also showed congestion of the periphery.

Conclusions. (1) The pulmonary circulation in certain types of heart disease and in certain stages of circulatory failure may show severe disturbance with a simultaneous normal state of the peripheral circulation. (2) Orthopnea and dyspnea may be present with congestive disturbances of the pulmonary circulation alone and with normal peripheral venous pressure. (3) Auricular fibrillation with the same degree of increased venous pressure had a tendency to be associated with lower cardiac output than heart disease with regular rhythm. (4) Digitalis and rest may improve patients with orthopnea and pulmonary congestion without *necessarily* changing the cardiac output. In such cases it has been observed that the mean pulmonary velocity of blood flow increased even if the maximal velocity was unaltered. Notwithstanding the lack of essential change in cardiac output, the distribution curve of the dye in the pulmonary circuit assumed a steeper, narrower, and more symmetrical character, indicating a more normal pressure relation in the pulmonary circuit even in the presence of unaltered cardiac output. (5) In the group of patients with auricular fibrillation with low cardiac output, the increase in the volume and velocity of blood flow following digitalis and rest was particularly prominent. (6) Simultaneously with the improvement of the mean velocity of the blood flow in the pulmonary circulation, there is a decrease in the residual air and an increase in the vital capacity, indicating a dependence of the proper function of the pulmonary alveoli on the state of the pulmonary circulation. (7) These observations are in accord with the concept of the mechanism of circulatory failure expressed in a previous communication.[7]

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[7] Weiss, Soma, *Ann. Int. Med.*, 1931, **5**, 100.

6298

Effect of Potassium Chloride on Rate of Penetration of Dyes.

MARIAN IRWIN.

From the Laboratories of The Rockefeller Institute for Medical Research.

A previous paper[1] has shown that KCl decreases the rate of penetration of dyes into the sap of *Nitella.* (1) The results with a cell model, consisting of a non-aqueous substance placed between cresyl blue solution and the artificial sap, show that when chloroform is used the rate is not decreased when 0.1 M KCl is added to the sap, but when aniline is employed it is decreased somewhat. (2) The same difference is observed when chloroform and aniline are shaken with 0.1 M KCl and then placed between the 2 aqueous phases when the sap contains no KCl. (3) When *o*-anisidine in chloroform is employed as the membrane, cresyl blue can be made to penetrate into the sap rapidly whether the sap contains KCl or not, provided the external pH value is considerably higher than the internal, but KCl has some inhibitory effect. An acid dye like phenol red is capable of penetrating the sap rapidly whether the sap contains KCl or not.

In dealing with the rate of penetration of substances into the sap in the model it is essential to consider several properties of the constituents of the non-aqueous layer such as chemical constitution, acid or basic character, dissociation constant, and solubility in water.

6299

Importance of the Internal Phase Boundary in Penetration of Dye into the Vacuole.

MARIAN IRWIN.

From the Laboratories of The Rockefeller Institute for Medical Research.

A cell model[1] was used consisting of chloroform placed between the dye solution and the artificial sap. R_o = the rate of penetration of dye from the dye solution into the chloroform, and R_s = the rate of penetration of dye from the chloroform into the sap. These are obtained by determining the concentrations of dye in the aqueous phases during one hour. The absorption coefficients of the dye are determined by shaking chloroform with the aqueous solutions. These are C_o = conc. of dye in the chloroform/conc. of dye in the dye solution, and C_s = conc. of dye in the chloroform/conc. of dye in the sap.

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[1] Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 54.

[1] For description see Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 135.

(1) The absorption coefficients of crystal violet do not differ very much between pH 5.5 and pH 9, and the rate of penetration of dye into the sap does not vary to any extent in this range. This is because the dye exists chiefly in one form.

(2) The absorption coefficient of methyl violet varies roughly from 4 at pH 5.5 to 20 at pH 9. This is due to the fact that methyl violet is a mixture. (*a*) When $C_o = C_s = 4$ the rates R_o and R_s are comparatively rapid. (*b*) When $C_o = 20$ and $C_s = 4$ there is an increase in R_o and R_s, but this is very small compared to the increase in C_o. (*c*) When $C_o = 4$ and $C_s = 20$, the change in R_o may be considered negligible while the decrease in R_s is roughly proportional to the increase in C_s as compared to the results in (*a*). (*d*) When $C_o = C_s = 20$, R_o is slightly higher than in (*a*), but the increase is very small in comparison with that in C_o, while the decrease in R_s is roughly proportional to the increase in C_s.

(3) The absorption coefficient of methyl violet increases approximately from 4 to 371 when 0.1 M KCl is added to the dye solution at pH 5.5. The rate of penetration of dye into the sap is greatly reduced when the sap contains 0.1 M KCl, but the presence of the same amount of the salt in the dye solution does not affect the rate to the same extent. The rate of penetration of dye into the chloroform from the dye solution is not appreciably affected by the presence of salt in the dye solution or in the sap. These results confirm those on crystal violet.[2]

In the cell model the rate of penetration of dye into the sap appears to be influenced more by the alteration in the absorption coefficient at the internal phase boundary than at the external phase boundary.

In these experiments, volume relations, thickness of the chloroform layer, distance between the phase boundaries, and rate of stirring play important parts. The fact that R_o is not greatly affected by the alteration in C_o may be partly due to the volume relations. Experiments are in progress to determine this point.

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[2] Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 993.

6300

Immunity Phenomena in Transmissible Leucosis of Fowls.*

J. FURTH.

From the Henry Phipps Institute, University of Pennsylvania.

The discoverer of transmissible leucosis, Ellermann, thought that there is no immunity to leucosis because fowls resisting one inoculation may succumb to reinoculation. More recent knowledge gained on immunity against Rous tumors (Rous, Mottram, Andrewes, Mueller, etc.) necessitates a revision of this conclusion.

Active immunity. Cell-free virus and leucemic cells must be considered separately, because leucemic cells behave like neoplastic cells and specific immunity is not known to develop in tumor-bearing animals. Furthermore the virus itself may be protected from immune bodies by association with cells. Accordingly 8 possibilities were tested with the following results:

TABLE I.

Material first injected	Result	Material reinjected	No. fowls injected	No. transmissible leucosis	No. lymphoid leucosis
Cell-free	Negative	Cell-free	16	6	1
,, ,,	Leucosis, recovery	,, ,,	4	0	0
,, ,,	Negative	Cell-containing	8	4	1
,, ,,	Leucosis, recovery	,,	7	3	0
Cell-containing	Negative	Cell-free	3	0	0
,, ,,	Leucosis, recovery	,, ,,	4	0	1
,, ,,	Negative	Cell-containing	4	0	1
,, ,,	Leucosis, recovery	,, ,,	10	1†	0

† Succumbed to leucosis after 6 reinjections with whole leucemic blood.

The fowls resistant both to cell-free virus and leucemic cells are those that were previously injected with material containing live leucemic cells. Their resistance may in part be due to antibodies against the free virus; antibodies active against leucemic cells have however thus far not been demonstrated. Resistance to reinjection with cells if not caused by acquired immunity, may be either an inherited character becoming manifest through elimination of susceptible fowls by the first injection, or a non-specific resistance-increase due to the first injection, or to other conditions, such as advancing age.

The reinjections were made with 0.5 to 1 cc. of leucemic blood

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* This investigation has been supported by a Fund for the Study of Leucemia and Related Diseases. Mr. Charles Breedis assisted in the work.

or plasma, estimated to contain 10,000 to 1,000,000 infective doses. It is possible that smaller amounts may reveal some immunity in groups 1, 3, 4. Among the 56 fowls tested, 4 had lymphoid leucosis (as compared to 14 with transmissible leucosis). They were grouped as negative, because according to previous investigations lymphoid leucosis is not caused by the agent of transmissible leucosis.

Passive immunity. When leucemic plasma of fowls was mixed with the serum of presumably immune fowls and injected within about 20 minutes, a mild but distinct protection was observed. Of the 27 fowls thus inoculated 26% developed leucosis as compared with 40% of the 15 fowls given a similar mixture containing normal instead of immune serum. Protection was complete against at least 1000 M.L.D. when mixtures of virus and immune serum were incubated at 37.5°C. for one hour, as shown in the following experiment.

Of 4 fowls given 2.5 cc. of a mixture of 3 cc. normal serum plus 0.5 cc. leucemic plasma, 3 developed leucosis after 21 days. Of 4 fowls receiving 2.5 cc. of the same normal serum plus 0.0005 cc. of leucemic plasma 3 developed leucosis, but after 40 days. All of 8 fowls receiving similar mixtures containing immune instead of normal serum remained free from leucosis. The immune serum was obtained from fowls resisting several inoculations of whole leucemic blood.

Protection against material containing leucemic cells on the contrary thus far has not been demonstrated. In 5 experiments involving 23 fowls inoculated with a mixture of immune serum plus leucemic cells, 61% of the former group developed leucosis, and 53% of the latter.

Failure to produce virus-free leucosis in the fowl. The experiments described indicate that fowls develop antibodies against the cell-free virus. The possibility suggests itself that virus-free leucosis might be produced by introducing leucemic cells, which are apparently neoplastic, into fowls immune to the virus.

With this in view a fowl found resistant to the free virus was injected with leucemic cells. As soon as its blood indicated incipient leucosis plasma and cells were injected each into 3 chickens. One of each group developed leucosis. Two months later, when the disease was advanced, this test was repeated in 8 fowls, half of which were injected with plasma, half with cells; all developed leucosis.

This experiment suggests that the filterable agent when in association with cells is protected against antibodies and that during cell-division it is handed over to the daughter cells unharmed by viricidal bodies. A repetition of the last experiment awaits fowls highly immune to the virus, but susceptible to leucemic cells.

6301

Readily Acid-Hydrolysable Phosphorus of the Blood in Infants with Rickets.

HARRY BAKWIN, OSCAR BODANSKY AND REUBEN TURNER.

From the Children's Medical Service and Department of Pathology, Bellevue Hospital, and the Department of Pediatrics, New York University.

The P fraction in red blood cells, readily hydrolysable by N mineral acid, was estimated in non-rachitic and rachitic infants.

The method of Lohmann[1] for this fraction in muscle, as adapted by Barrenscheen and Vasarhelyi[2] for blood, was used with slight modification. One volume of blood was added to 4 volumes of 10% trichloracetic acid and the mixture filtered. Ortho-phosphate was determined by the method of Fiske and Subbarow.[3] Two cc. of 2 N HCl were added to two cc. of the filtrate, the mixture was heated at 100°C. for 10 minutes and the P again determined. From the amount of P freed in this way, the amount of ortho-phosphate and the hematocrit reading, the whole blood and red cell content of this fraction was calculated.

Variations in time of heating from 10 to 15 minutes gave no significant difference in the amount of P freed. No difference was noted between the use of H_2SO_4 and HCl as the hydrolyzing acids. This fraction is gradually split in the trichloracetic acid filtrate, from 0.5 to 1.0 mg. being freed in the first 24 hours at ice-box temperature. It does not give the color reaction for pyro-phosphate described by Davenport and Sacks.[4]

The results in non-rachitic and rachitic children are shown in Tables I and II. The values have been calculated for whole blood and for the red cells. Values for the ortho-phosphate content of serum, whole blood and red cells are included in the tables.

The average value for this fraction in the whole blood of non-rachitic children was 5.20 mg. % as P, with a standard deviation of 0.86 mg. % and a coefficient of variation of 16.5%. The average

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[1] Lohmann, K., *Biochem. Z.*, 1928, **202**, 466.

[2] Barrenscheen, H. K., and Vasarhelyi, B., *Biochem. Z.*, 1931, **230**, 330.

[3] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

[4] Davenport, H. A., and Sacks, J., *J. Biol. Chem.*, 1928, **81**, 469.

TABLE I.
Blood P Fraction, Readily Hydrolysable by N Mineral Acid, in Non-rachitic Infants.

Case No.	Red cells vol. %	Mg. P per 100 cc.				
		Serum	Whole Blood		Red Cells	
		Ortho-phosphate	Ortho-phosphate	Acid hydrolysable phosphate	Ortho-phosphate	Acid hydrolysable phosphate
1	—	—	3.7	4.0	—	—
2	—	—	4.0	5.8	—	—
3	27	6.2	6.0	5.2	5.4	19.2
4	42	—	4.4	7.6	—	18.1
5	41	—	5.0	5.6	—	13.7
6	49	—	4.8	5.8	—	11.9
7	49	—	4.3	5.8	—	11.9
8	44	—	4.0	4.5	—	10.2
9	38	5.0	3.9	5.7	2.1	15.0
10	41	—	3.3	5.9	—	14.4
11	36	—	4.8	4.6	—	12.8
12	50	—	3.3	7.0	—	14.0
13	41	—	3.6	5.0	—	12.2
14	—	—	5.3	5.7	—	—
15	—	—	3.8	4.9	—	—
16	29	—	4.3	4.8	—	16.6
17	41	—	4.5	5.6	—	13.7
18	—	—	4.0	5.3	—	—
19	37	4.7	4.0	4.1	3.0	11.1
20	32	5.6	4.2	4.3	1.3	13.5
21	30	4.7	4.1	4.3	2.7	14.3
22	31	4.6	3.7	4.1	1.7	13.3
23	33	6.0	4.6	5.4	1.7	16.2
24	37	5.5	4.3	4.4	2.3	11.9
25	—	6.0	4.6	4.9	—	—
26	37	3.0	2.5	4.8	1.6	13.0
27	34	5.0	4.1	5.1	2.4	15.0
28	37	6.5	4.8	5.7	1.9	15.5
29	29	6.7	5.0	3.9	0.8	13.5
30	34	5.5	4.2	4.5	1.6	13.2
31	32	5.8	4.9	4.7	3.0	14.7
32	34	6.5	5.4	5.9	3.3	17.4
33	37	4.7	4.1	3.8	3.1	10.3
34	38	5.3	4.5	6.4	3.2	16.8
35	40	—	4.6	5.4	—	13.5
36	—	5.2	3.9	4.8	—	—
Average		5.41	4.36	5.20	2.48	14.10
Standard deviation		0.87	0.65	0.86	1.08	2.19
Coefficient variation %		16.0	14.8	16.5	43.4	15.5

Nos. 9, 10, 12, 13, 17, 19 were older children.

value for the red cell content was 14.10 mg. % with a standard deviation of 2.19 mg. % and a coefficient of variation of 15.5%.

The average value for the whole blood of rachitic children was 3.76 mg. % as P with a standard deviation of 0.69 mg. % and a coefficient of variation of 18.4%. The average value for the red cell content was 11.40 mg. % with a standard deviation of 1.89 mg. % and a coefficient of variation of 16.6%.

The P fraction, readily hydrolyzed by N mineral acid, is reduced in the blood of rachitic children.

TABLE II.
Blood P Fraction, Readily Hydrolysable by N Mineral Acid, in Infants with Rickets.

		Mg. P per 100 cc.				
		Serum	Whole Blood		Red Cells	
Case No.	Red cells vol. %	Ortho-phosphate	Ortho-phosphate	Acid hydrolysable phosphate	Ortho-phosphate	Acid hydrolysable phosphate
1	36	4.4	4.1	3.9	3.6	10.8
2	30	1.6	1.0	2.3	—0.4	7.7
3	32	3.1	2.2	3.8	0.3	11.9
4	24	3.1	2.9	3.3	2.3	13.8
5	29	3.0	2.4	3.6	1.0	12.4
6	33	5.1	3.3	4.5	—0.3	13.5
7	33	—	2.1	4.4	—	13.2
8	33	2.4	1.6	3.9	0.0	11.7
9	26	2.1	1.2	3.2	—0.2	12.3
10	44	1.8	1.3	5.0	0.7	11.4
11	28	1.4	1.2	2.8	0.7	10.0
12	28	3.0	2.7	3.6	1.7	12.8
13	33	5.3	4.4	4.2	2.7	12.6
14	35	3.1	2.3	4.7	0.9	13.4
15	41	2.4	1.7	2.8	0.7	6.8
16	33	3.6	2.4	3.1	0.0	9.3
17	35	1.7	1.3	3.4	0.6	9.7
18	39	1.7	1.4	4.6	1.0	11.8
19	33	3.3	2.6	3.8	1.2	11.4
Average		2.95	2.28	3.76	1.03	11.40
Standard deviation		1.14	1.24	0.69	1.05	1.89
Coefficient variation %		38.7	54.3	18.4	102.0	16.6

No. 10 was an older child.

6302

Experimental Production of Chronic and Sub-acute Adrenal Insufficiency in Dogs and Cats.

J. M. ROGOFF.

From the Laboratory of Experimental Medicine, Western Reserve University.

Chronic adrenal insufficiency, clinically and pathologically comparable with Addison's disease, has not heretofore been successfully produced in experimental animals. This has been attempted by various procedures, especially excision or denervation of the adrenals, destruction by mechanical, thermal, bacterial or chemical agents and by radiation, and ligation of blood vessels.

In 1917, I performed a number of experiments, upon cats and dogs, in which I practised mass ligation, ligation of the adrenal vein and ligation of the lumbo-adrenal vein on both sides of the gland. In general, my results were similar to those of others.[1, 2]

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[1] Torrini, U. L., *Sperim.*, 1910, **64**, 342.

[2] Hartman, F. A., and Blatz, W. E., *Endoc.*, 1919, **3**, 137.

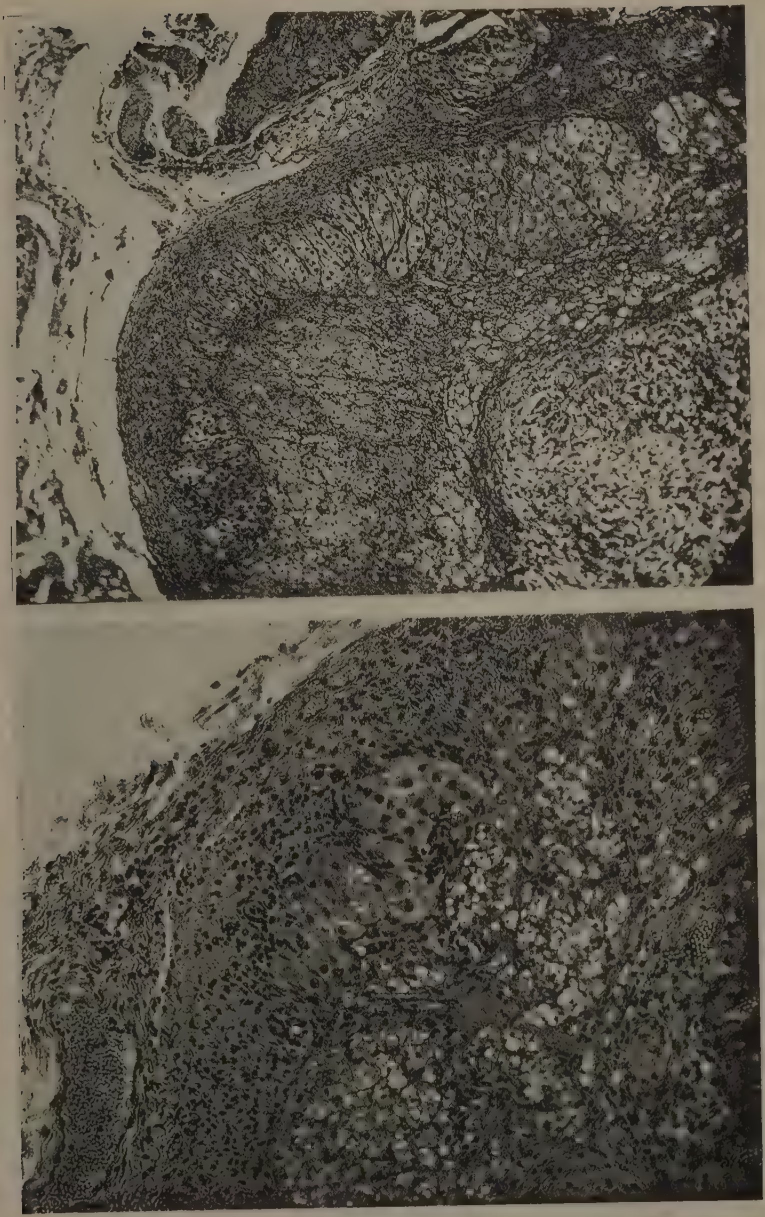

FIG. 1.

Photomicrographs of sections of cat's adrenals showing (left gland, above) small zone of surviving (regenerated) cortical tissue, degenerated and necrotic cortex, well preserved medulla, 83 ×; right gland (below) nodule of regenerated cortical cells, lymphocytic infiltration and fibrosis of cortex, degenerated and necrotic cortex, well preserved medulla, 180 ×.

I have observed a number of cases of Addison's disease among which were found at autopsy, pathological changes in the adrenal glands similar to those described by Kovács,[3] as "Cytotoxische Schrumpfnebenniere" and "Vaskuläre Schrumpfnebenniere". The excellent discussion by Kovács, together with my observations on adrenal insufficiency in laboratory animals and on Addison's disease, renewed my interest in the possibility of inducing circulatory and other changes in the adrenals which might lead to chronic insufficiency.

Physiological and clinical observations suggested the possibility that certain types of adrenal atrophy, associated with Addison's disease, may be results of the immediate effects of circulatory disturbances associated with thrombosis and anemic infarction. Accordingly, attempts were made to create comparable lesions by *subtotal* ligations of adrenal blood vessels. This was accomplished, sometimes in a single operation (bilaterally, or unilaterally after excision of the opposite gland), sometimes following the first operation by one or more subsequent operations, to create additional circulatory disturbances by ligating vessels of the collateral circulation (which often become enlarged after the first operation). A number of animals developed clinical manifestations of sub-acute and chronic adrenal insufficiency, in most instances comparable with Addison's disease. The physiological and anatomical significance of the collateral venous anastomoses in relation to the adrenals has been considered by Cow[4] and others. Its relation to thrombosis of the suprarenal vein has been studied by Seecof.[5]

Survival periods of the animals, in the group observed, range from about 2 to 14 months. Among those surviving long periods we have observed acute phenomena (anorexia, vomiting, asthenia and nervous symptoms) at intervals and finally an acute condition which terminates fatally. During these periods of acute (or subacute) adrenal insufficiency we have observed blood changes similar to those occurring in adrenalectomized animals, though apparently less severe. Laparotomy, performed during the survival of some animals, revealed the same remarkable congestion of the pancreas observed by us following double adrenalectomy.

At autopsy, the adrenal glands showed various degrees and extent of degeneration and necrosis. In most cases, the pathological changes in the adrenals consisted of *selective* degeneration of the cortex, the medulla remaining intact (in others the medulla shared in extensive degeneration and necrosis) and zones of regenerated cortical cells were present, presenting a picture (Fig. 1) comparable, if not identical, with that seen in so-called "cytotoxic atrophy" associated with Addison's disease.

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[3] Kovács, W., *Ziegler's Beitr.*, 1928, **79**, 213.

[4] Cow, D., *J. Physiol.*, 1914, **48**, 443.

[5] Seecof, D. P., *Proc. N. Y. Path. Soc.*, 1926, **26**, 126.

In a few experiments it was possible to create pathological changes in the adrenals leading to purulent infiltration or areas of purulent necrosis, by superposing infection on the degenerative processes induced by sub-total ligation. Frequently, this procedure leads to total acute purulent necrosis of the gland.

In some experiments the glands were removed for examination during periods of good health, in others when clinical symptoms were present. These and other observations are still in progress. The experiments thus far performed constitute a foundation for studies which may lead to a better understanding of Addison's disease and of the function of the adrenal cortex.

It is a pleasure to acknowledge my gratitude to Doctors D. P. Seecof and H. Goldblatt for valuable aid in preparation and interpretation of much of my experimental and human pathological material.

6303

Studies in Hypertension. I. Injection of Various Substances, Especially Amino-Acids, upon Blood Pressure of Rabbits.*

HENRY A. RAFSKY, A. BERNHARD AND G. L. ROHDENBURG.
(With the technical assistance of Dorothy Davis.)

From the Achelis Laboratory, Lenox Hill Hospital, New York City.

The smallest dose of the selected substances which would produce histological changes in the kidneys of rabbits was first determined. Doses approximately one-half this amount were then injected daily intramuscularly and the effect upon the blood pressure studied. Uranium nitrate, lead acetate, phosphorus, ergot, viosterol, cholesterol, lecithin, putrescin, tyramine, arginine carbonate, asparagine, glutamic acid, histidine, tyrosine, tryptophane, succinic acid, aspartic acid, and guanidine carbonate were used. Of this entire group aspartic acid and guanidine alone produced progressive increase in systolic blood pressure.

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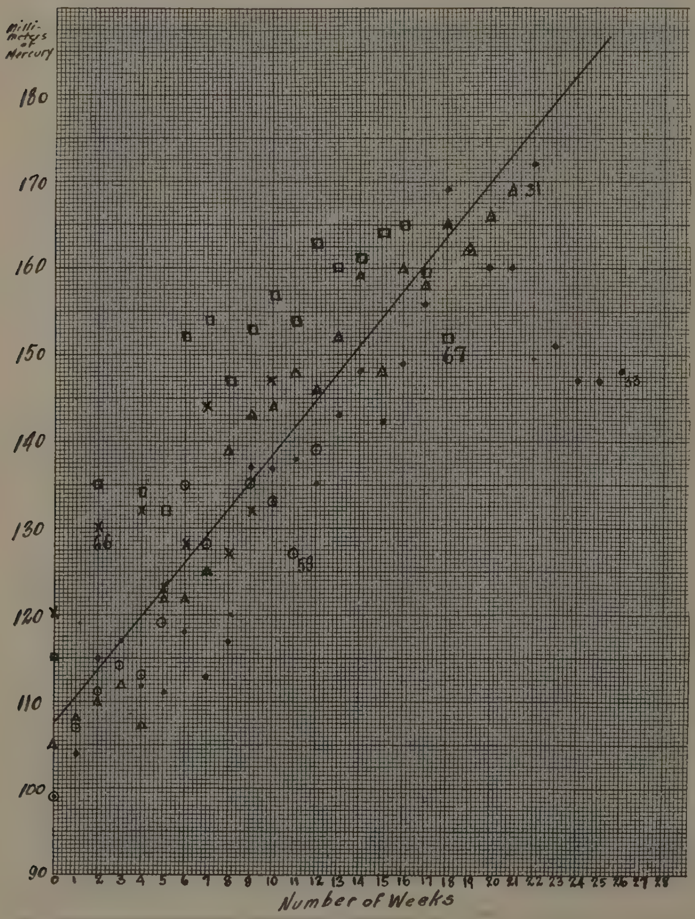
* Aided by a grant from The W. C. Durant Fund for the Study of Hypertension.

The animals were kept in the laboratory for at least one week before blood pressures were taken. Then daily blood pressures were recorded for at least one week before the animals were used. The animals received the same diet of oats, greens, and carrots. The control animals were observed for the same length of time as treated ones. Only the systolic pressure was taken and the procedure of McGregor[1] used. The blood pressure was taken at first daily, and

AVERAGE WEEKLY BLOOD PRESSURE. ASPARTIC ACID GROUP



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[1] McGregor, L., *Arch. Path.*, 1928, **5**, 630.

then 3 times weekly, the longer period being found sufficient for statistical purposes. The pressures for each week were averaged and the rise or fall recorded. Increases of 20 mm. of mercury or greater were considered as significant.

Fourteen animals injected with neutralized aspartic acid (doses varying between 20 and 250 mg. per kilo of body weight) all showed a rise in systolic blood pressure, the maximum rise being 25 mm. after 6 weeks and 73 mm. after 22 weeks. (See chart 1). The minimum dose of aspartic acid to produce an elevation of blood pressure was 40 mg. per kilo. Further increases of aspartic acid did not cause a more rapid or higher elevation of blood pressure. Blood urea and creatinin were within normal limits. The urine showed albumin and casts in 4 animals. Newburgh and Marsh[2] report that aspartic acid is mildly nephrotoxic when injected intravenously into rabbits. They also state that the smallest dose that produced renal injury was 0.5 gm. per kilo. Our dosage was considerably less. The histological changes observed were thickening of the elastic tissues of the aorta, congestion of the glomeruli of the kidney, and in a few instances tubular degeneration.

Two rabbits injected with guanidine carbonate, neutralized with HCl, showed a maximum rise of 42 and 46 mm. in systolic blood pressure after 5 weeks. No abnormal changes in the blood urea or creatinin or in the urine were noted. No histological change in the kidney was observed in one animal after 7 weeks of injection.

Rabbits injected with glutamic acid and succinic acid showed no definite rise in blood pressure.

6304

Studies in Hypertension. II. Changes in Blood Proteins in Experimental Hypertension.

ADOLPH BERNHARD AND I. J. DREKTER.

From the Achelis Laboratory and the A. Jacobi Division for Children, Lenox Hill Hospital, New York City.

It has been shown[1] that in rabbits the daily injection of aspartic acid and guanidine produces a definite rise in blood pressure. The blood proteins of these animals were analyzed both before and after a definite and prolonged elevation of the blood pressure was obtained. Control animals injected with putrescin, tyrosine, and tryptophane were observed for a similar length of time and their blood proteins were analyzed.

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[2] Newburgh, L. H., and Marsh, P. L., *Arch. Int. Med.*, 1925, **36**, 682.

[1] Rafsky, H. A., Bernhard, A., and Rohdenburg, G. L., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 1243.

The red cells were separated from the oxalated blood by hypertonic sodium sulphate as suggested by Folin[2], and the protein precipitated from the supernatant fluid with sulphuric acid. This procedure precipitates all the serum proteins from a definite volume of blood. The Van Slyke[3] method of protein analysis was adapted to micro methods.

The whole blood, the red cells, and the serum proteins from 1 cc. of blood were hydrolyzed with 10 cc. of concentrated HCl in a small Erlenmeyer flask covered with a funnel and placed on a sand bath. The hydrolysis was continued for 4 hours and the mixture evaporated to dryness on the water bath. The contents of the flask were diluted to 15 cc., transferred to a large centrifuge tube and magnesium hydroxide added, then aerated for 1 hour for the determination of the amide nitrogen. The residue is centrifuged, filtered, and washed, and the filtrate and washings diluted to 40 cc. Five cc. is removed for the determination of nitrogen after hydrolysis, and to the remaining filtrate, 5 cc. of 10% phosphotungstic acid in 10% H_2SO_4 is added, the mixture allowed to stand overnight, then centrifuged and washed with phosphotungstic acid solution. The entire precipitate obtained by centrifugation was digested and the total nitrogen determined, which gives basic amino nitrogen precipitated by phosphotungstic acid. The filtrate from the phosphotungstic acid precipitation was extracted with amyl alcohol and ether, neutralized with NaOH, 10 cc. removed for total nitrogen, and 2 cc. for the manometric determination of monoamino nitrogen.

Osborne and Harris[4] and Van Slyke[3] have shown that under suitable conditions the solubility of the phosphotungstic acid precipitate is very small. We have observed that the precipitation of basic amino nitrogen by phosphotungstic acid is much more complete in solutions diluted as stated above. The results reported in this communication represent the total nitrogen of the basic amino nitrogen precipitated, duplicate determinations checking within 0.1 mg. of nitrogen. Therefore it seems that differences obtained in this fraction between normal rabbits and those in which hypertension has been induced are significant.

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[2] Folin, O., *J. Biol. Chem.*, 1930, **86**, 173.

[3] Van Slyke, D. D., *J. Biol. Chem.*, 1911, **10**, 15.

[4] Osborne, T. B., and Harris, I. F., *J. Am. Chem. Soc.*, 1903, **25**, 323.

TABLE I.

Blood serum proteins of normal and hypertensive rabbits in mg. per 100 cc.

Blood Pressure M. M. of Hg.	Total N.	Amide N.	Humin N.	Total N.* of Filtrate	Basic Amino N precipitated by Phosphotungstic Acid	Total N in Phosphotungstic Acid Filtrate	Monoamino N.	Remarks.
102	700	27	123	550	140	410	400	Control Animal
110	630	17	93	520	110	400	380	Control Animal
98	830	20	280	530	100	400	400	Control Period
104	830	20	260	550	130	420	400	After Tryptophane injections
100	900	24	336	540	120	420	400	Control Period
102	900	24	326	550	140	400	400	After Tyrosine injections
106	800	23	203	570	100	470	400	Control Period
108	800	20	210	570	130	440	400	After Putrescine injections
90	850	21	289	540	110	430	400	Control Period
130	850	21	199	630	30	600	600	After Aspartic Acid injections
107	900	22	348	530	130	400	300	Control Period
128	780	22	368	390	40	350	340	After Guanidine injections
104	800	40	260	500	100	400	350	Control Period
140	850	40	170	640	40	600	600	After Guanidine injections

*From hydrolysis after removal of humin and amide N.

The completeness of the hydrolysis of the proteins was determined by experiment for periods of 2, 4, 8, and 24 hours and checked against Van Slyke's procedure. It was found that there was no further hydrolysis at the end of 24 hours than at the end of 2 hours, but to insure complete hydrolysis of the various blood samples the 4-hour period was employed.

The formation of humin nitrogen from the basic amino nitrogen is rather small. Roxas[5] showed that less than 10% of the basic amino nitrogen played any rôle in humin nitrogen formation. In the results reported here the differences in basic amino nitrogen between normal and hypertensive animals is far too great to be explained by the possibility of humin nitrogen formation from this fraction.

In the control animals there were no appreciable changes in the total nitrogen, amid nitrogen, or amino-nitrogen fractions or a rise of blood pressure after injection of putrescin, tryptophane, or tyrosin.

In those animals in which hypertension was produced by the injection of aspartic acid and guanidine very definite changes were observed in the blood serum fraction. (Table I.)

The basic amino nitrogen precipitated by phosphotungstic acid is about 20% of the total nitrogen after hydrolysis for normal animals, and the monoamino nitrogen is between 70-80%. In those animals with hypertension the basic amino nitrogen varied between 4.9-10%, while the monoamino nitrogen varied between 80-90%. There were no changes in the amide nitrogen either before or after injection of aspartic acid or guanidine.

6305

Studies in Hypertension. III. Blood Proteins in Children with Hypertension.

ADOLPH BERNHARD, JEROME S. LEOPOLD AND I. J. DREKTER.

From the Achelis Laboratory and the A. Jacobi Division for Children, Lenox Hill Hospital, New York City.

In rabbits with hypertension changes in the basic amino nitrogen, and the monoamino nitrogen fractions of the blood proteins have been described by Bernhard and Drekter[1]. The investigation has been extended to include a study of the blood of normal children and the blood of children and adults with hypertension.

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[5] Roxas, M. L., *J. Biol. Chem.*, 1916, **27**, 71.

[1] Bernhard, A., and Drekter, I. J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1245.

Fifteen normal children between the ages of 17 months and 12 years served as controls. In this group the basic amino nitrogen content of the whole blood varied between 370 and 620 mg. per 100 cc., and the monoamino nitrogen between 1200 and 1990 mg. per 100 cc. Considered in per cent of the total nitrogen after hydrolysis the basic amino nitrogen of the whole blood varied between 19.4% and 27.0%, while the monoamino nitrogen varied between 62 and 79%. The basic amino nitrogen in the red cells varied between 280 and 490 mg. per 100 cc., and the monoamino nitrogen between 900 and 1650 mg. per 100 cc. The percentage of the total nitrogen after hydrolysis ranges between 17.8% and 27.8% for the basic amino nitrogen. The basic amino nitrogen of the serum protein is fairly constant from 100-150 mg. per 100 cc. and the monoamino nitrogen is between 300 and 420 mg. per 100 cc. The variation in per cent of total nitrogen after hydrolysis varies between 20.0% and 27.8% for the basic amino nitrogen, and between 55.5 and 77% for the monoamino nitrogen.

The blood proteins were analyzed in 4 cases of hypertension. One case, a child, 4 years of age, had a blood pressure of 170/86 mm. The other 3 cases were adults, with blood pressures of 170/95, 230/124, and 210/125 respectively. In all of these cases the blood urea and creatinine were within normal limits.

In all 4 cases of hypertension there was a marked decrease in the basic amino nitrogen precipitated by phosphotungstic acid, of the serum protein fraction of the blood. The values for this fraction varied 60 and 80 mg. per 100 cc., while the per cent of the total nitrogen after hydrolysis varied between 10.3 and 15.0%. There was an increase in the monoamino nitrogen fraction.

6306

Comparative Toxicity of Nicotine Alkaloid and Nicotine Salts.

DAVID I. MACHT AND LYMAN C. CRAIG.*

From the Laboratory of Organic Chemistry, Johns Hopkins University, and Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc.

The idea is generally accepted by most pharmacologists that the physiological activity and toxicity of powerful alkaloids are practically the same as that of their salts, provided allowance is made for the slight increase in molecular weight of the salts due to the acid radicles, and for the difference in solubility between the alkaloids and their salts. The chief exception to the rule, in a limited way, is cocaine. The addition of sodium bicarbonate to cocaine solution increases its efficiency as a local anesthetic when applied directly to nerve trunks, and this is claimed to be due to the greater penetration of the alkaloid for the nerve tissue. Such a difference is not noted after hypodermic or intradermal injections.[1, 2, 3]

In connection with a comparative pharmacological and toxicological study of β-pyridyl-α(N-methyl)-pyrrolidine, or nicotine, and a new series of related chemical derivatives of pyrrolidine, the writers noted a marked difference in the physiological activity of the alkaloid itself and its salts. Previous writers have noted a difference in toxicity between nicotine and its salts but their work dealt mostly with the effects of these chemicals as insecticides. Del Quercio,[4] reporting some experiments on silkworms sprayed with aqueous solutions of nicotine and its salts, states that acidulated nicotine was less active than the alkaline substance itself. Moore and Graham,[5] comparing the effects of nicotine sulphate with and without sodium carbonate on certain aphids, found that the sulphate was less active than nicotine solution containing sodium carbonate. Moore and Row[6] found nicotine itself more toxic for the frog than nicotine hydrochloride. De Ong[7] showed that free nicotine administered orally to chickens was more toxic than nicotine sulphate; and Langley and Dickinson noted that nicotine solution was more active for ganglion cells and nerve fibers than nicotine sulphate.[8] Richardson and Shepard found that the toxicity of nicotine for mosquito larvae varied to some extent with the hydrogen ion concentration.[9]

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*National Research Fellow.

[1] Gros, *Arch. f. exp. Pathol. u. Pharmakol.*, 1910, **62**, 380.

[2] Laewen, *Münch. med. Wchnschrft.*, 1910, **57**, 2044.

[3] Sollmann, *J. Am. Med. Assn.*, 1918, **70**, 216.

[4] Del Guercio, *Nuove Relaz. R. Staz. Ent. Agr. Firenze,* 1900, **1**, 124.

[5] Moore and Graham, *J. Agr. Research,* 1917, **10**, 47.

[6] Moore and Row, *J. Physiol.*, 1897, **22**, 273.

[7] De Ong, *J. Econ. Ent.*, 1923, **16**, 486.

This difference in activity between the alkaloid and its salts was explained either by the variation in the hydrogen ion concentration of the solutions or by the volatility of nicotine itself and its consequent rapid diffusion through the bodies of the insects. These explanations may hold good for experiments on insects and other small organisms, but it was deemed desirable to determine definitely whether a difference between the nicotine alkaloid and its salts may be demonstrated in large animals and also in living plants.

A nicotine of the highest chemical purity was prepared by one of the authors, and solutions of its various salts were so made as to contain exactly the same amount of nicotine by weight, irrespective of anions. The following 5 salts were studied: hydrochloride, acetate, salicylate, sulphate and tartrate. Phytopharmacological studies were made of the toxicity of the various compounds for the seedlings of *Lupinus albus* suspended in dilute solutions of the drugs; and the zoöpharmacological activity of the compounds was studied on goldfish, *Carassius aureus;* minnows, *Gambusia;* larvae of tadpoles, *Rana sylvatica,* 4 weeks old, and *Rana catesbiana,* 1 year old; leeches, *Hirudo medicinalis;* small crayfish, *Cambarus;* frogs, *Rana clamata;* white mice, white rats, canaries and on cats. The results are exhibited in Tables I and II.

Without exception there was a marked difference in toxicity between the nicotine alkaloid and its salts for both animals and living plants. Obviously, this difference in toxicity cannot be explained on the basis of volatility of the nicotine alkaloid as has been suggested in case of sprays for insecticidal purposes, nor by variations in the hydrogen ion concentration of the solutions used, especially when the drugs were injected in such animals as rats and mice intramuscularly or intraperitoneally, and intravenously in cats. The concentrations in which the seedlings of *Lupinus albus* were suspended were made by dissolving the drugs in Shive solution,[10] containing magnesium sulphate, diacid potassium phosphate and calcium nitrate. Studies on the hydrogen ion concentration of such solutions of nicotine and its salts in this plant physiological medium showed very little difference in their reactions. In Table II it will be noted

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[8] Langley and Dickinson, *Roy. Soc. (London) Proc.*, 1889, Ser. B 46, 423.

[9] Richardson and Shepard, *J. Agr. Research,* 1930, **41**, 337.

[10] Shive, *Physiol. Researches,* 1915, **1**, 327.

TABLE I.

Toxicity of Nicotine and its Salts for Animals.

Animals	Nicotine Alkaloid Concentration	Nicotine Alkaloid Dead	Nicotine Salts	Nicotine Salts Concentration	Nicotine Salts Dead
Carassius aureus	1:5,000	3 min.	N. hydrochloride	1:5,000	12 min.
			N. acetate	1:5,000	18 min.
			N. salicylate	1:5,000	11 min.
	1:10,000	4 min.	N. sulphate	1:5,000	13 min.
			N. tartrate	1:5,000	4½+ hr.
Gambusia	1:10,000	2 min.	N. hydrochloride	1:5,000	4 min.
Larvæ of *Rana sylvatica,* 4 weeks old			N. hydrochloride	1:10,000	2+ hr.
			N. acetate	1:10,000	2½+ hr.
			N. salicylate	1:10,000	18 hr.
			N. salicylate	1:5,000	2½ hr.
			N. sulphate	1:10,000	2½+ hr.
	1:10,000	9 min.	N. tartrate	1:10,000	18 hr.
Larvæ of *Rana catesbiana,* 1 year old			N. hydrochloride	1:5,000	65 min.
			N. salicylate	1:5,000	29 min.
	1:5,000	3 min.	N. sulphate	1:5,000	49 min.
Hirudo medicinalis	1:10,000	6 min.	N. hydrochloride	1:5,000	12 min.
Cambarus	1:10,000	53 min.	" "	1:5,000	113 min.
Rana clamata	0.5 mg. per 30 gm.; paralysis and characteristic posture in 25 min.		" "	0.5 mg. per 30 gm., little or no effect in 25 min.	
White Mice	0.1 mg. per 10 gm.; dead in 3 min.		" "	0.1 mg. per 10 gm.; dead in 6 min.	
	0.1 mg. per 20 gm.; dead in 4 min.		" "	0.1 mg. per 20 gm.; dead in 10+ min.	
White Rats	M.l.d., 27 mg. per kilo		" "	recovery after 27 mg. per kilo	
Canary	0.05 mg.; immediate convulsions		" "	0.05 mg., little or no effect	
Cats	Intravenous injection under ether, 1:2,000 solution gives m.l.d., 2 mg. per kilo		" "	intravenous injection under ether of 1:2,000 solution at same rate gives m.l.d., 2.6 mg. per kilo	

TABLE II.

Toxicity of Nicotine and Its Salts for Lupinus Albus.

Nicotine	Index of Growth in Concentration 1:5,000 %	Index of Growth in Concentration 1:2,000 %
Alkaloid	52	34
Acetate	69	50
Hydrochloride	81	50
Salicylate	26	10
Sulphate	90	56
Tartrate	85	80

that the salicylate of nicotine was much more toxic for plant tissues than other salts. This agrees with the findings of Macht and Livingston on the great toxicity of benzoates for living plants,[11] which observation was later found to hold good also for salicylates. The marked difference between the alkaloid nicotine and its salts is especially interesting when compared with the relative effects of certain other alkaloids and their salts, which were used as controls. We have made comparative pharmacological studies with solutions of atropine and atropine sulphate, cocaine and cocaine hydrochloride and strychnine (alcoholic solutions) and strychnine sulphate and found that those alkaloids did not differ substantially in toxicity from their salts.

6307

The Critical Period for Control of Sex in Moina.

A. M. BANTA AND C. A. STUART.

From the Biological Laboratory, Brown University.

Banta and Brown[1] have shown that with crowding under favorable food conditions the critical period for sex control with *Moina macrocopa* is approximately 4 hours before the eggs are laid into the brood chamber. Stuart, Cooper and Miller,[2] while unable to affect control of the sex of the same Cladoceran species later than the fourth hour before egg laying, concluded that sex could be influenced by conditions prevailing previous to the fourth hour before egg laying. The following experiment was designed to show that the critical period for sex control is dependent to some extent upon the environmental conditions under which the animals are reared, *i. e.,* upon environmental conditions present earlier than, and interrupted before, the time of the critical period as described by Banta and Brown for crowding under good food conditions.

In 4 series of bottles, A, B, C and D, each series containing from 15 to 20 bottles, were placed 75 ml. of varying dilutions of manure infusion and 10 young *Moina macrocopa* females. The infusion in series A and B was diluted 12.5 times, in series C, 25 times, and in

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[11] Macht and Livingston, *J. Gen. Physiol.*, 1922, **4**, 573.

[1] Banta, A. M., and Brown, L. A., *Proc. Nat. Acad. Sci.*, 1929, **10**, 71.

[2] Stuart, C. A., Cooper, H. J., and Miller, Rose, *Roux. Arch.*, Abt. D, 1932, **126**, 325.

D, 100 times. (Dilutions were made with pond water.) All bottles were then incubated at approximately 25°C. Series A served as a control and was not manipulated in any way. Approximately 15 hours before the egg-laying period of the mothers in series B, C, and D the crowded mothers from one bottle in each series were isolated, each mother being transferred to a separate bottle containing 25 ml. of normal medium (concentrated manure infusion diluted 12.5 times). One hour later the same procedure was repeated with another bottle from each series and again another 1 hour later, and so on every hour until the mothers in the different series laid eggs.

By this procedure, in series B for example, mothers were subjected to a male-inducing condition (crowding) up to periods of time varying from 18 to 1 hour before egg-laying when the crowded mothers were transferred to a female-determining condition, *i. e.,* isolated in an adequate amount of fresh culture medium. Obviously then, the time before egg laying at which isolating the mothers failed to overcome the effect of crowding will constitute the critical period for sex control in the environment specified. In these experiments, which involved the sexing of more than 13,000 young, it was found that the critical period for sex control in these animals was different for the different environments.. In series B, medium diluted 12.5 times, it was the same as that previously determined by Banta and Brown—approximately 4 hours before egg-laying. In series C, however, in which the medium was diluted 25 times, the critical period was found to be much earlier, namely from 7 to 8 hours before egg laying. The critical period for sex control in series D (medium diluted 100 times) appeared to be as early as 13 to 14 hours before egg laying. Furthermore, it was found in this series that while mothers isolated from 6 to 14 hours before egg-laying produced males, mothers isolated from 1 to 5 hours before egg laying produced, for the most part, female young. This is not surprising in view of the fact that Stuart, Tallman and Cooper[3] have shown that *Moina macrocopa* mothers reared in very dilute medium up to the time of release produce only female young. The mothers in control series A (medium diluted 12.5 times as in series B) which remained crowded until releases occurred produced at least some males in every experiment, showing that the male production and the observed critical period in series B, C and D were the result of controlled environment rather than chance.

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[3] Stuart, C. A., Tallman, Juanita, and Cooper, H. J., *Physiol. Zool.*, 1931, 4, 594.

The critical period for sex control in this animal requires further elucidation. As Banta and Brown[1] used the term it meant the time before egg laying at which a mother must be under a specific (crowded) environment in order to affect the sex of her young. This seems a legitimate use of the term, critical period. There is presumptive evidence that the actual *sex determination* is chromosomal. Allen and Banta[4] obtained evidence that the nuclear spindle for the single maturation division begins to form roughly 1½ hours before the eggs are laid. It seems probable that the influential external environmental factors must have produced their effect within the egg at about that time. Whether this effective condition is a state of nutrition, accumulation or removal of some substance, or level of metabolism (or something else) is beside the present point.

Banta and Brown[5] have reported that when temperature is used as the controlling factor the temperature condition must operate for a long period (during most of the previous life of the mother) in order to be most effective upon the sex of the offspring. The present data likewise indicate that environmental conditions operative many hours before egg laying are effective upon the sex of the young. If the term, critical period, is to continue to be used to designate the time at which a specific environmental condition must be operative, it, obviously, is not a fixed and definite time within the life of the individual mother but is the critical period for the operation of a particular external environmental condition.

On the other hand the critical period, theoretically, might be defined with reference to the immediate *internal* environment of, or the condition of, the egg itself, *i. e.,* as the time at which the effects produced by the specific external environmental conditions must have become built up (and remain potent) within or immediately about the egg itself.[6] This period, as just defined, should theoretically be the same regardless of the influential environmental factors employed.

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[4] Allen, Ezra, and Banta, A. M., *J. Morph. and Physiol.*, 1929, **64**, 123.

[5] Banta, A. M., and Brown, L. A., *Yearbook Carn. Inst. of Wash.*, 1924, No. 23, 36. Brown, L. A., and Banta, A. M., *Physiol. Zool.*, 1932, **5**, 218.

[6] Banta, A. M., and Brown, L. A., *Yearbook Carn. Inst. of Wash.*, 1929, No. 28, 51.

6308

Food and Crowding as Sex Controlling Factors in Cladocera.

C. A. STUART AND H. J. COOPER. (Introduced by A. M. Banta.)

From the Biological Laboratory, Brown University.

In an attempt to show that crowding and the subsequent accumulation of excretory products were not essential for the production of males by Cladocera, Stuart and Cooper[1] reared isolated Moina mothers in from 100 to 500 ml. of very dilute manure infusion medium.[2] It was found that isolated mothers reared in as large a volume as 500 ml. of suitably diluted medium not infrequently produced males. It seemed of interest then to ascertain whether or not crowded mothers reared in exceedingly small volumes of concentrated or heavily bacterized medium[3] would produce female young.

After many preliminary experiments, the following procedure was selected: Manure infusion medium[3] or manure infusion to which filtrate of mascerated Cladocera ("cell juice") had been added was dispersed in 2 ml. amounts in 15x5 mm. vials. Ten young sister Cladocera *(Moina macrocopa)* were placed in each vial. The cell juice was prepared by collecting several thousand Cladocera with embryos or eggs in the brood chamber and grinding them up in a mortar in a small amount of pond water, then filtering the resulting extract through paper. The medium was bacterialized by the addition of one drop of a concentrated suspension of *Aerobacter aerogenes.*[3] The bacterization was repeated at from 4 to 8 hour intervals until the animals laid eggs, at which time the 10 mothers were placed in 100 ml. of normal culture medium to release their young. As controls 10 mothers, sisters of those in the vials, were reared in the usual 100 ml. of unbacterized medium in the usual 6 oz. bottles. When the mothers released, the number and sex of the young were determined. Twenty-six such experiments were carried out.

Mothers in the control bottles, reared in approximately 10 ml. of medium per mother, produced an average of 12.3 young per mother, of which 27.9% were males. The percentages of male young in the various bottles varied from 8% to 55%. On the other hand, the mothers reared until eggs had been laid in approximately 0.2 ml.

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[1] Stuart, C. A., and Cooper, H. J., *Physiol. Zool.*, 1932, **5**, 70.

[2] Banta, A. M., *Science* N. S., 1921, **53**, 557.

[3] Stuart, C. A., and Banta, A. M., *Physiol. Zool.*, 1931, **4**, 72.

of medium per mother, produced an average of 13.1 young per mother, of which but 6.3% were males. The percentages of male young in the various experimental vials ranged from 0% to 21%.

A most interesting fact brought out by these experiments is that the mothers in 5 of the vials produced an average of 14.0 young per mother of which 100% were females. This would seem to indicate that the detrimental effect of crowding and excretory substances had been entirely overcome in 5 cases by the addition of an adequate amount of food. The importance of the food factor in these experiments will be realized when one considers that Cooper, Stuart and Miller[4] have shown that 9 mothers reared in 2 ml. of unbacterized medium in similar vials, though they may survive for relatively long periods of time, rarely produce any young at all. It has been shown, therefore, that in certain cases, even with an extreme of crowding but with an adequate supply of living bacteria, Cladoceran mothers may produce female young.

The addition of "cell juice" appeared to favor the growth of Cladoceran mothers, as animals reared simply in bacterized manure infusions did not seem to thrive as well. We are inclined, however, to believe that the "cell juice" possessed little if any nutritive value for the Cladocera, but that it did furnish an excellent substrate for the growth of the added bacteria.

6309

Depression Periods in Cladocera.

C. A. STUART. (Introduced by A. M. Banta.)
From the Biological Laboratory, Brown University.

For more than 3 years the writer has been interested in the periodic depressions that so frequently occur in stock cultures of Cladocera reared in manure infusions. Such periods have been reported by Warren,[1] Hartman,[2] and Allee.[3] Stuart, Cooper and Coady[4] believed that no one environmental factor was responsible for their occurrence and suggested several factors that might be associated with the phenomenon. Since Allee[3] has so clearly shown the detrimental effects of unconditioned water on aquatic animals it occurred to the writer that this factor might play some rôle in depression periods in Cladocera.

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[4] Cooper, H. J., Stuart, C. A., and Miller, R., *Anat. Rec.*, 1932.

[1] Warren, E., *Quart. J. Microsc. Soc.*, 1900, **43**, 199.

[2] Hartman, O., *Arch. f. Zellforsch.*, 1919, **15**, 1.

[3] Allee, W. C., Animal Aggregations, Univ. Chicago Press, 1931.

[4] Stuart, C. A., Cooper, H. J., and Coady, H., in press.

Accordingly, during a part of the summer of 1931 when stock animals showed continued depression periods the pond water employed in the preparation of medium for these animals was treated in the following way. Five gallons of the raw water was filtered through paper and together with a suitable quantity of garden soil was put into an aquarium. A few hours later, after sedimentation of the gross silt, the aquarium was stocked with 100 or more adult Cladoceran mothers *(Moina macrocopa)*. In each of 6 separate experiments many of these animals died within 12 to 18 hours, and a similar number of mothers with eggs or embryos present in the brood chamber were added to the aquarium. For the most part these animals survived and released their young. The water was arbitrarily held to be conditioned in from 48 to 72 hours when a slight opalescence or turbidity appeared: this opalescence was shown by bacteriological counts to be due to the increased number of bacteria. The water was then filtered through paper and used for diluting a portion of the concentrated manure infusion employed for rearing these animals.[5] A portion of the same concentrated infusion was also diluted with unconditioned pond water from the same lot as that used for conditioning. Single Moina sisters were placed in each of 2 bottles, one containing 100 ml. of the medium prepared from conditioned water, the other a similar amount of medium made from unconditioned water. If the mothers survived to release, the number of young released and the number of second eggs laid by the mothers were noted. Six series of experiments were carried out, each series consisting of from 12 to 25 separate experiments.

Of 108 mothers reared in the conditioned medium 102 survived to release 1074 young and to lay subsequently a total of 1173 eggs for the second brood. In the unconditioned medium, however, only 83 of the 108 mothers survived to release 591 young and to lay 489 second eggs. The mortality in the conditioned medium was but 5.55% while in the unconditioned medium it was 23.14%. Moreover, in the conditioned medium the average number of young and second eggs per mother was 10.52 and 11.50 respectively, while in the unconditioned medium the average young and second eggs per mother was respectively 7.12 and 5.89.

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[5] Stuart, C. A., Tallman, J., and Cooper, H. J., *Physiol. Zool.*, 1931, 4, 581.

Sister lines of the stock Moina mothers during this depression period were, therefore, reared for several weeks in both conditioned and unconditioned media. It was evident that the mothers in the conditioned medium produced more young and more second eggs at all times than the sister mothers in the unconditioned medium. The conditioned medium failed, however, in rejuvenating completely the stock from the depression period.

6310

Effects of Administration of Cortin to the Hypophysectomized Rat.*

WAYNE J. ATWELL.

From the Department of Anatomy, University of Buffalo School of Medicine.

Among the results of experimental hypophysectomy in mammals are loss of weight, or, in young animals, failure to increase in weight, loss of *libido sexualis,* cessation of estrus, or sexual infantilism, lessened spontaneous activity, lowered metabolism and lowered temperature.[1, 2, 3] Autopsy of such animals shows atrophy of the thyroid, the adrenal cortex, the gonads, the secondary sex organs, and the thymus. It is apparent that the hypophysectomized animal is characterized not only by absence of the pituitary but also by a marked reduction in size, and presumably also in function, of several other highly important endocrine glands. Considerable light has been thrown on the respective rôles of the thyroid and the gonads in this complex endocrine disturbance.[2, 4] The functional relationships of the adrenal cortex in the above pictured syndrome are not so well known; what symptoms and what glandular deficiencies, if any, are secondary to the atrophy of this important endocrine gland following removal of the pituitary?

We have previously reported the results of cortin treatment in hypophysectomized Amphibia.[5] In our mammalian experiments albino rats weighing from 150-200 gm., principally from the Wistar

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* Aided by a grant from the Carnegie Corporation to the Carnegie Institution of Washington.

[1] Smith, P. E., *J. Am. Med. Assn.*, 1927, **88**, 158.

[2] Foster, G. L., and Smith, P. E., *J. Am. Med. Assn.*, 1926, **87**, 2151.

[3] Richter, C. P., and Wislocki, G. B., *Am. J. Physiol.*, 1930, **95**, 481.

[4] Smith, P. E., *Am. J. Physiol.*, 1932, **99**, 349.

[5] Atwell, W. J., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 621.

experimental colony strain were used. Beginning about the 6th day following hypophysectomy certain animals received daily two 0.5 cc. injections of cortin. Other operated animals were injected at similar times with an equal amount of salt solution. Still other litter mates, unoperated, were available for controls. The operated animals were weighed and daily vaginal smears obtained from the females. In the latter part of the experiment spontaneous activity cages, which recorded revolutions, were available and some of the animals lived in them. The rats were killed from 4 to 8 weeks following operation after having received treatment for from 3 to 7 weeks. Unoperated litter mates were killed at the same time. At autopsy, gonads, genital tract exclusive of gonads, adrenals, thyroids, thymus, liver, spleen and kidneys were carefully dissected out, weighed, and fixed in Bouin's fluid for histological study. The hypophysial region also was preserved, decalcified and sectioned serially. This procedure was deemed necessary in all cases to prove the success or failure of the operation. Twenty rats were hypophysectomized. Nine of these received cortical extract.

Cortin treatment failed to repair the atrophy of the gonads, the thyroids or the adrenal cortex. The interrupted oestrous cycles of the females were not restored. There was no restoration of the growth rate.

In certain respects, however, the cortin-treated rats were strikingly different from the untreated. Instead of being cold, apathetic and almost entirely inactive as hypophysectomized animals characteristically are, they were distinctly active and showed a body temperature slightly under normal.

These observations provide an experimental basis for the expectation[6] that cortical extract will relieve the asthenia, hypothermia, and presumably also the hypotension, of Simmond's disease. They likewise provide a necessary link in the evidence that the relief of myasthenia which follows administration of hypophysial growth hormone to hypophysectomized rats (Evans and coworkers[7]) results from activation of the adrenal cortex.

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[6] Calder, R. M., *Bull. Johns Hopkins Hosp.*, 1932, **50**, 87.

[7] Evans, H. M., Meyer, K., Pencharz, R., and Simpson, M. E., *Science*, 1932, **75**, 442.

6311

Note on the Serological Differentiation of Steric Isomers.

K. LANDSTEINER and J. VAN DER SCHEER.

From the Laboratories of the Rockefeller Institute for Medical Research, New York City.

A relationship between spatial configuration and serological specificity has been established in several instances.[1, 2, 3, 4] In recent studies it has been found that the cis-trans isomerism of maleïc and fumaric acid can be detected serologically. The immune serum used was obtained by injection of an antigen consisting of protein to which was coupled diazotized p-aminosuccinanilic acid. The reaction of this immune serum with the corresponding antigen was inhibited by succinic acid (as sodium salt) and moreover by maleïc, but not by fumaric acid. This differentiation of the cis- and trans-forms extended to the monoesters of the two acids and furthermore to the methyl substituted compounds (citraconic and mesaconic acid).

On the basis of our former results the suggestion was made[5] that in certain cases serological reactions could be used for determining spatial configuration, in a way similar to that in which enzymes have been applied to this purpose. At the same time a first example was cited dealing with the correlation between the optically active forms of malic and tartaric acids. Since no details have been given, a description of the experiments will follow.

For the tests immune sera were used specific for levo- and dextro-tartaric acids, prepared as described previously[2] and in addition an immune serum specific for levo-malic acid. This was obtained by injections of rabbits with an antigen made by coupling diazotized levo-para-aminomalanilic acid to horse serum. Levo- and dextro-rotatory para-aminomalanilic acids were prepared by reduction of the corresponding nitro-compounds.* For the preparation of these compounds the two optically active malic acids were condensed with para-nitraniline.* Which of the two possible levo- or dextro-nitranilides the substances isolated represent remains to be determined;

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[1] Landsteiner K., and Van der Scheer, J., *J. Exp. Med.*, 1928, **48**, 315.

[2] Landsteiner, K., and Van der Scheer, J., *J. Exp. Med.*, 1929, **50**, 407.

[3] Avery, O., and Goebel, W. F., *J. Exp. Med.*, 1929, **50**, 521.

[4] Avery, O., Goebel, W. F., and Babers, F. H., *J. Exp. Med.*, 1932, **55**, 761.

[5] *Naturwissenschaften*, 1930, **18**, 563.

**cf.* [2].

but it seems probable that the condensation would take place more readily with the carboxyl next to the hydroxyl group. For the experiment, this point is of no significance because in both levo-compounds the arrangement of the groups around the asymmetric carbon atom would correspond to one optically active form of tartranilic acid and in both dextro-compounds to the other.

Levo-para-nitromalanilic acid. The crude substance, obtained by fusion of levo-malic acid and p-nitraniline, was recrystallized twice from water and 25% alcohol. Microscopic needles. Melting point: 168-169° C. Titration: 0.127 gm. dissolved in 80% alcohol, required for neutralization 5 c.c. of N/10 NaOH. Formula $C_{10}H_{10}O_6N_2$ requires 5 c.c. A 1% solution in methyl alcohol gave at 25° a rotation of —0.77 in a 2 d.m. tube with sodium light $[\alpha]_D - 38.5$.

Dextro-para-nitromalanilic acid. The crude substance obtained by fusion of dextro-malic acid[6] and para-nitraniline was recrystallized twice from water and 25% alcohol. Microscopic needles. Melting point: 168-169° C. Titration: 0.127 gm. dissolved in 80% alcohol, required for neutralization 4.95 c.c. N/10 NaOH. Formula $C_{10}H_{10}O_6N_2$ requires 5 c.c. A 1% solution in methyl alcohol gave at 25° C. a rotation of +0.76 in a 2 d.m. tube with sodium light; $[\alpha]_D + 38$.

Levo-para-aminomalanilic acid. The crude material was crystallized from 8 volumes of hot water. Fine microscopic needles. The substance became dark but did not melt when heated to 230° C. Kjeldahl nitrogen determination after drying at 100° *in vacuo;* $C_{10}H_{12}O_4N_2$ calculated N 12.5%, found N 12.44%. An aqueous solution containing 0.1 gm. of the substance and 0.6 c.c. of normal HCl in a volume of 5 c.c. gave at 25° C. a rotation of —1.18 in a 2 d.m. tube; $[\alpha]_D - 29.5$.

Dextro-para-aminomalanilic acid. The crude material was crystallized from 8 volumes of hot water. Fine microscopic needles. The substance became dark but did not melt when heated to 230° C. Kjeldahl nitrogen determination after drying at 100° *in vacuo*: $C_{10}H_{12}O_4N_2$ calculated N 12.5%, found N 12.7%. An aqueous solution containing 0.1 gm. of the substance and 0.6 c.c. of normal HCl in a volume of 5 c.c. gave at 25° C. a rotation of +1.2 in a 2 d.m. tube; $[\alpha]_D + 30$.

An immune serum for levo-malanilic acid reacted on the levo- and not on the dextro-tartranilic antigen.

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[6] **Dakin, H. D., *J. Biol. Chem.*, 1924, 59, 7.**

TABLE

For a description of the technique we refer to a former publication[2]. The readings were taken after 2 hours at room temperature. The antigens were used in a dilution of 1:500 of a 5 per cent solution.

Immune sera for azo-proteins made from horse serum and	Antigens prepared from chicken serum and:			
	levo-p-amino-tartranilic acid	dextro-p-amino-tartranilic acid	levo-p-amino-malanilic acid	dextro-p-amino-malanilic acid
levo-p-amino-tartranilic acid	+±	0	+	tr
dextro-p-amino-tartranilic acid	0	+±	0	+

From the tests one may conclude that there is a correlation between the tartaric and malic acids which rotate polarized light in the same sense. Freudenberg and Brauns[7] correlated d-tartaric acid to d-malic acid by converting the former into monoacetyl-tartaric acid-dimethylester, acetyl-chloromalic acid-dimethylester, chloromalic acid and finally into d-malic acid. The fact that the same result has been arrived at by the use of immune sera seems to confirm the applicability of the serological method to questions of spatial configuration.

6312

Production of Experimental Endometrial Hyperplasia.*

J. M. WOLFE, MARY CAMPBELL AND JOHN C. BURCH.

From the Departments of Anatomy and Gynecology, Vanderbilt University School of Medicine.

Endometrial hyperplasia has been known in this country since 1900, when it was described by Cullen[1]. Schroeder's[2] conclusion that endometrial hyperplasia was due to changes in the ovary resulting from a deficiency of the corpus luteum has been amply con-

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[7] Freudenberg, K., and Brauns, F., *Ber. D. Chem. Ges.*, 1922, **55**, 1339.

*We are greatly indebted to Parke, Davis and Company and to E. R. Squibb and Son for the œstrus-producing extracts used in these experiments.

This work has been supported by a grant from the National Research Council Committee on Problems of Sex.

[1] Cullen, T. S., *Cancer of the Uterus*, Philadelphia, 1900.

[2] Schroeder, Robert. *Arch. f. Gynak.*, 1921, **98**, 81.

firmed. Fluhmann[3], and Burch, Williams, and Cunningham[4] have studied the condition from the hormonal standpoint, and consider that the endometrial pathology is due mainly to the action of oestrin. While in their experiments[4] the cellular changes of endometrial hyperplasia were faithfully reproduced, the characteristic gland pattern was lacking. This gland pattern, the most striking feature of the condition, was considered less important than the cellular changes. The characteristic gland pattern was experimentally produced and recognized as such by Hofbauer[5]. He repeatedly implanted anterior lobe substance in guinea pigs with intact ovaries. It is impossible to evaluate the relative effects of hypophyseal and ovarian hormones, as the formation of the ovarian hormones would be stimulated. The inference is clear that oestrin must be the major factor, as the hormone of the corpus luteum exercises little or no effect on an endometrium unless sensitized by oestrin.

To test further this conception of the etiology of endometrial hyperplasia, a series of 24 spayed guinea pigs and 20 spayed rats were given varying amounts of oestrin over relatively long periods of time. Sections of the uteri of these animals were removed at stated intervals during the injection period.

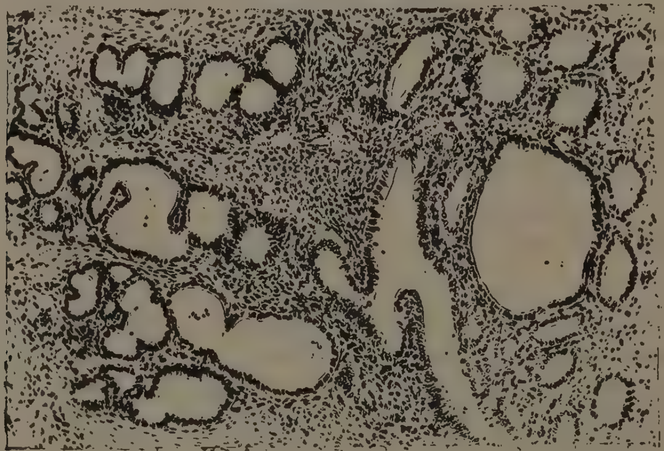

FIG. 1.

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[3] Fluhmann, C. F. *Surg. Gyn. and Obstet.*, 1931, **52**, 1051.

[4] Burch, J. C., Williams, W. L., and Cunningham, R. S. *Surg. Gyn. and Obstet.*, 1931, **53**, 338.

[5] Hofbauer, J., *Surg., Gyn. and Obstet.*, 1931, **52**, 222.

A large percentage of the guinea pigs which received the oestrin injections exhibited uteri in which the cellular and glandular picture was identical with that found in human hyperplasia. The glands, many of which were cystic, were very prominent throughout the greatly thickened mucosa. Figure 1 shows that these specimens were quite similar to the typical Swiss-cheese pattern found in human cases. As a rule, this picture was found in the uteri of guinea pigs which had received from 10 to 50 units of oestrin daily for 8 to 15 days.

In guinea pigs it was possible to induce both the glandular and cellular change of hyperplasia of the endometrium in all of the animals injected. In rats, the cellular changes were easily induced, but we were able to produce cystic dilatation of the glands in only 40% of the animals studied.

6313

Bactericidal Property of an Ultra-Violet Irradiated Petrolatum-Lanolin Mixture.

A. TRELOAR ROSS. (Introduced by H. J. Sears.)

From the Departments of Bacteriology and Surgery, University of Oregon Medical School.

Remarkable results on purulent wounds following the use of ultra-violet irradiated petrolatum, reported by Eising,[1] prompted us to determine whether such petrolatum had any bactericidal effect *in vitro*. We used a mixture of 2 parts petrolatum molle and one part lanolin, the latter being added to increase tissue absorption of the irradiated petrolatum.

Technic. A 2:1 petrolatum-lanolin mixture was melted and rendered sterile by infra-red heat. Twenty cc. of the mixture were placed in sterile petri dishes and irradiated directly for varying periods of time by a Burdick ultra-violet lamp at a distance of 12 inches. At the end of the time allotted for exposure 1 cc. of a normal saline suspension of organisms was thoroughly mixed with the petrolatum-lanolin. At varying intervals thereafter one loopful of the inoculated mixture was seeded on agar plates and the growth noted. For control we used: (1) Non-irradiated petrolatum-lanolin to which organisms had been added; (2) Petrolatum-lanolin to which organisms had been added before irradiation; (3) Petrolatum-lanolin which was irradiated for the same varying periods of time as the directly irradiated samples but with the petri dish covers left on to filter out the shorter ultra-violet rays as much as possible. The organisms used were *Staphylococcus aureus* and *Bacillus pyocyaneus.*

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[1] Eising, E. H., *Annals Surg.*, 1931, **43**, 123.

Staph. aureus was practically always killed within 24 hours by a 2-hour directly irradiated mixture. *B. pyocyaneus* gave less consistent results, but was usually killed within 24 hours by the 4-hour directly irradiated mixture. The plates irradiated with their covers on showed no bactericidal property except those exposed for 4 hours, which consistently killed *Staph. aureus* within 24 hours, thus suggesting that over the prolonged exposure time sufficient rays passed through the glass to irradiate the mixture. The non-irradiated controls gave abundant growth, while those to which organisms had been added before irradiation gave no growth.

Time and facilities allowed only one test to determine how long the irradiated mixture could be effective, and this test showed that a 4-hour directly irradiated mixture one week old killed *Staph. aureus* and *B. pyocyaneus* within 24 hours.

That the effect of irradiation is bactericidal rather than bacteriostatic was shown by making broth cultures from the irradiated mixtures which had given no growth on agar and also from the non-irradiated controls. Warm broth was used on the theory that the grease would be melted, freeing the bacteria. Here again the non-irradiated controls gave growth while the irradiated mixtures did not.

We can only surmise the nature of the bactericidal agent in the irradiated petrolatum-lanolin. Montgomery[2] has shown that irradiated ergosterol is not bactericidal; hence the ergosterol in the lanolin of our mixture can be excluded as an active agent. Our non-irradiated controls show that the infra-red used in sterilization of the mixture plays no rôle. The fact that irradiation turns colorless petrolatum yellow suggests a change in molecular arrangement of that substance, but it is doubtful if such a chemical change renders the petrolatum capable of killing bacteria. A more appealing theory is that secondary ultra-violet emanations are held by the petrolatum-lanolin. The ultra-violet spectrum is between 1850 and 4000 Angstrom units. It is plausible to suppose that the secondary rays retained by the petrolatum-lanolin mixture are similar to the longer rays of the biologically active or so-called "vital" portion of the ultra-violet spectrum. This view is supported by the results of

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[2] Montgomery, B. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 481.

Eising, who produced stencil designs on hermetically sealed X-ray films placed over irradiated petrolatum in a light-tight box, and by French.[3]

I wish to express my gratitude to Dr. Arthur C. Jones of the division of Physical Therapy for his valuable suggestions.

6314

Further Purification of the Adrenal Cortical Hormone.*

J. J. PFIFFNER, H. M. VARS, P. A. BOTT AND W. W. SWINGLE.

From the Biological Laboratory, Princeton University.

In studying the problem of further purification of the adrenal cortical hormone we have used the following 3 types of fractionation procedure on the active material obtained from whole beef adrenal glands by the usual permutit fractionation: (1) distribution between an immiscible solvent and aqueous alkali, (2) distribution between an immiscible solvent and aqueous acid, and (3) fractionation with organic solvent mixtures. The starting material and the various fractions obtained were assayed on adrenalectomized dogs by the technique previously described.[1]

Four hundred and seventy mg. of alcohol-soluble fraction obtained from 4000 gm. of beef adrenal glands and containing 8000 D. U. (dog units) were dissolved in ether and washed with 0.05 N NaOH. The aqueous alkaline solution was washed with fresh ether and the ether solutions combined. The ether-soluble fraction (200 mg.) was transferred to water (70 mg. water-soluble) for assay and was found to contain less than 500 D. U. The alkaline washings were adjusted to pH 5.6 and on assay contained between 500 and 1000 D. U. In this manipulation, therefore, about 6500 D. U. were apparently destroyed.

The above procedure was repeated on another aliquot of 470 mg. containing 8000 D. U. except that 0.05 N HCl was used instead of alkali. The ether soluble fraction (350 mg.) contained between 500 and 1000 D. U., while the aqueous acid washings after adjustment to pH 5.2 assayed between 6000 and 7000 D. U. The

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[3] French, F. S., *Clifton Med. Bul.*, 1932, **18**, 21.

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation of New York.

[1] Harrop, G. A., Pfiffner, J. J., Weinstein, A., and Swingle, W. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 449.

cortical hormone can apparently be transferred to aqueous acid without destruction. This fractionation step is being used in further studies.

One of the most potent fractions thus far obtained was prepared by hexane fractionation. An aliquot of 470 mg. containing 8000 D. U. was dissolved in 10 cc. of absolute ethyl alcohol and precipitated by the gradual addition of 9 volumes of hexane. The supnatant fluid was decanted, the precipitate dissolved in 5 cc. of alcohol and precipitated as before. The hexane-alcohol-soluble fraction (370 mg.) was transferred to water. The water-soluble fraction (150 mg.) assayed 8000 D. U. or approximately 50 D. U. per mg. of solids.

6315

Actions of Dinitrophenol.

W. C. CUTTING and M. L. TAINTER.

From the Division of Neuropsychiatry of the Department of Medicine, and the Department of Pharmacology, Stanford University School of Medicine.

Following the demonstration by C. Heymans[1] and others that α-dinitrophenol (1-2-4) produces fever in experimental animals, we have attempted to determine the cause of the rise of temperature, the effects on various functions, and its possible therapeutic applications.

Dinitrophenol produces fever in pigeons, rats, rabbits, cats, dogs, and man, in doses of 5 to 40 mg. per kilo, the exact dose depending on the species and route of administration. After subcutaneous or intramuscular injection, the temperature begins to rise in about 10 minutes and steadily increases until the maximum of up to 4°C. increase is reached, in from one-half to 4 hours. Then there may be a subsidence of the fever with complete recovery, or, if a fatal dose has been given, a sudden stoppage of respiration and circulation, and death. Rigor sets in at once.

Preceding the onset of the fever, and throughout the entire febrile period, there is a marked respiratory stimulation, including both the rate and depth, so the total ventilation increases up to 10 times the resting values. The respiratory stimulation may be prevented or diminished by large doses of morphine. Conversely, dinitrophenol increases the ventilation to better than the normal values after it has been decreased by toxic or fatal doses of morphine, chloral, alcohol or barbital. The stimulation compares favorably with that caused by caffeine. The improvement in respiration does not prevent death, if otherwise fatal doses of these hypnotics have been given; thus indicating that the respiratory depression *per se* is possibly not the primary cause of death from the hypnotics. During the period of maximum respiratory stimulation, there is little or no change in blood pressure and pulse rate, and the sugar, pH and CO_2-combining power of plasma are unaltered. Hence the increase of temperature and respiration is not accompanied by an acidosis or the appearance of appreciable amounts of fixed acid in the blood. The rise of temperature is not prevented by destruction of the brain and spinal cord or by complete curarization, so that the mechanism of production would appear to be a peripheral one. When fever-producing doses are given repeatedly to dogs at intervals of 3 days over a period of 6 weeks, constant febrile responses are elicited, with little or no evidence of toxicity, either grossly or on microscopic study of the tissues. The other available isomers of nitro-, dinitro- and trinitrophenol are less effective in producing fever, or cause it only in fatal doses. Even with the α-dinitrophenol the margin between the febrile and fatal dose is narrow.

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[1] Personal communication.

Experiments are being made to elucidate the mechanism of the fever production, the details of the pharmacologic action, and the cause of death, and to determine possible antidotes, and therapeutic usefulness, of this drug in man.

6316

Studies in Ageing Eggs. Changes in Permeability of Egg Membrane.

A. J. GOLDFORB AND VICTOR SCHECHTER.

From the Marine Biological Laboratory, Woods Hole, and the Biological Laboratory, College of the City of New York.

The volume of unfertilized eggs of *Arbacia punctulata* aged in sea water at 19 to 22° C., pH 8.2, is not constant. The volume increases slowly and progressively during approximately the first 23 hours after shedding. The increase in volume of eggs from different females ranges from 1.8 to 3.2%. With further ageing the volume decreases progressively.

Samples of such ageing eggs were placed at successive ages in the same concentration of dilute sea water, at 21.5°, pH 8.2, and the increase in volume of each egg measured during 5 to 7 successive minutes. Nine to 21 eggs were measured at each age. The average of these measurements at each age was plotted and the constant of the curve, *i. e.,* the permeability constant, K, was calculated by the method of Lucké and McCutcheon. K measured the amount of water passing through a unit of surface per unit difference of osmotic pressure per unit time.

TABLE I.

Showing increase in permeability (K) with increasing age of eggs. Calculations based on extrapolated and on average Vo volumes.

Age in hrs.	Permeability K Extrapolated Vo.	Permeability K Aver Vo.
1	.120	.121
3½	.134	.131
6	.132	.129
24	.139	.135
28	.171	.171
32	.167	.168
48	.166	.164
54	.162	.179
	Increase in permeability—24.2%.	

The volume of the eggs before immersion in the hypotonic solution was determined in 3 ways: (1) average of 50 measured eggs, (2) by extrapolation, (3) average of all extrapolated Vo values. The permeability constants were calculated by all 3 methods. The results, though varying, all agree in this, that a marked increase in permeability occurs with ageing of eggs.

The increase in permeability was 24.2, 41.0, 43.0, 47.0, and 68% in 5 different experiments, when 50 and 60% sea water was used. In one experiment with 80% solution of sea water the increase was 27%.

There appears in each experiment to be 3 stages in the increased permeability: (1) an initial low rate between 0 and 2 hours after shedding, (2) a higher rate between 3 and about 24 hours, (3) a highest rate between 24 and about 54 hours. These stages may represent 3 steps in a progressive increase of permeability with ageing of eggs.

6317

Studies in Ageing Eggs. Changes in Resistance of Egg Membrane to Bursting in Hypotonic Solutions.

A. J. GOLDFORB and VICTOR SCHECHTER.

From the Marine Biological Laboratory, Woods Hole, and the Biological Laboratory, College of the City of New York.

When unfertilized Arbacia eggs of one female were placed in the same hypotonic solution of sea water, at the same temperature and pH, the time required for 50% to cytolyze decreased sharply with ageing of eggs, i. e., the rate of cytolysis involving bursting of egg membranes increased rapidly with ageing of eggs.

TABLE I.

Time in min. for 50% of eggs to cytolyze in a 30% solution of sea water at 21.5.

Min.	Age Hr.
14½	1
13½	3½
7⅚	6½
5⅘	20
4½	24
4⅛	28
4	32
3⅔	44
2½	46½
3	49
2½	52
2³⁄₁₂	56
2⅓	58½
1⅚	68
2	73

Table I is an example of one experiment in which a 30% solution of sea water was used at 21.5° and pH 8.2. One hour after shedding, 50% burst in 14½ minutes. When 73 hours old, 50% burst in 2 minutes.

Similar striking increase in the rate of cytolysis with ageing of eggs occurred in every experiment in every concentration, viz., 0 (distilled water), 10, 20, 30, 45, and 60% solutions of sea water.

The curves representing the rate of cytolysis in different concentrations of sea water were not parallel. In more concentrated solutions the initial rates of cytolysis were very much slower, but the final rates approximate closely the rates in more dilute solutions.

When eggs from one female were subjected at each age to 3 different concentrations, for example, 20, 30, and 45% solutions of sea water, at the same temperature and pH, the 3 curves representing the rates of cytolysis in each solution converged with ageing of eggs. The terminal rates are closely correlated with the osmotic pressure differences across the egg membrane. The convergence indicates a greater effect of osmotic forces on cytolysis at later ages and, conversely, lesser effect at early ages.

The increase in surface area of eggs placed in a given hypotonic solution at constant temperature and duration, is greater with ageing of eggs. This is due to increased permeability of the egg membrane with age. The resulting per cent increase of stretching of the membrane was measured and compared with the percent increase in the rate of bursting. The 2 curves are not parallel. Bursting is progressively faster than stretching. Hence, increased stretching of membrane with ageing, is not solely responsible for the increased rate of bursting.

These 2 lines of evidence, namely, (1) convergence of bursting rates in different concentrations, (2) non-parallelism between rates of stretching of membrane and rates of bursting, strongly indicate a third factor, namely, a change (i. e., a decrease) in tensile strength of the egg membrane with ageing of eggs.

This increased stretching plus the decreased tensile strength of the egg membrane, we believe are the major factors involved in the more rapid bursting of the egg membrane during ageing.

6318

Further Study on the Incidence of B. dysenteriae in the Normal Individual.*

DONOVAN C. BROWNE. (Introduced by C. W. Duval.)

From the Department of Pathology, Tulane University, New Orleans.

Study of the stools of 50 healthy individuals, selected from various sections of New Orleans, was previously reported. *B. dysenteriæ* was recovered from the feces in 6 instances, or 12%. Of these 6 isolations, 5 were the lactose fermenting strain described by Duval[1], and one was the true Shiga type.

The present report completes the survey, which includes 50 additional cases. The first group was selected from the public clinics,

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*Aided by a grant from David Trautman Schwartz Research Fund.

[1] Duval, C. W., *J. Am. Med. Assn.*, 1904, **46**, 381.

the last group studied comprised cases selected from private practice. In the first group, the patients lived in various parts of the city, some under good, others under poor hygienic conditions; while those of the second group came from a less widely distributed area, and lived under much better hygienic conditions.

The same technique as previously used was employed. Fresh stool specimens were obtained by proctoscopic examinations, were cultured in dextrose bouillon for from 2 to 3 hours, plated, and *B. dysenteriæ* colonies subsequently searched for. The only variation from the previous technique was that the patients were given a mild laxative about 6 to 8 hours before the stool specimens were obtained, and in 3 instances, repeated examinations were made of the stool specimen.

From each of the normal cases, blood specimens were obtained for the purpose of testing for specific agglutinins. The organisms used in the agglutination test were *B. Flexner, B. Duval, B. Shiga, B. Hiss Y.* In those instances where *B. dysenteriæ* organisms were recovered, they were tested with the homologous serum and serum from the experimental immunized animal.

In 3 instances dysentery bacilli were isolated from the second group of 50 cases. This percentage is considerably less than in the first group selected from the public clinics. There is also a notable drop in percentage of the lactose fermenter, while *B. Hiss Y., B. Flexner,* were each encountered once. In previous studies carried out with dysenteric cases in the South, there has been a considerable number of *B. dysenteriæ* (*Flexner*) encountered and we might have expected to recover this organism more frequently.

In 2 cases the patient's serum agglutinated the isolated organism in a dilution of 1-100. In one case the isolated organism was agglutinated in a 1-25 dilution.

6319

Pathological Changes in Liver Accompanying Intestinal Obstruction and Strangulation.

WARREN H. COLE AND ROBERT ELMAN. (Introduced by Evarts A. Graham.)
From the Department of Surgery, Washington University Medical School, and Barnes Hospital, St. Louis, Missouri.

In an effort to determine whether or not a consistent significant damage in the liver is produced by intestinal obstruction and strangulation of the intestine, we produced these 2 conditions experimentally in 28 dogs by severing the intestine under aseptic technique and closing the ends, and by constriction of a loop (varying from 2 inches to 12 inches in length) of intestine so that the lumen of the bowel and the entire blood supply were obstructed, respectively. The animals with simple obstruction lived for a variable length of time or were killed, 1 to 12 days after operation. This variability introduces an error since the ones killed may have lived a day or 2 longer if left to die spontaneously. When a strangulation was produced, death took place in 24 to 48 hours with considerably more uniformity, depending directly upon the length of the strangulated loop.

There are few and conflicting reports on the effect of intestinal obstruction on the liver, Colp and Louria[1], Seulberger[2], Trinchera[3].

A definite and the most significant histological effect produced in the livers of dogs with simple intestinal obstruction consisted of central necrosis of the cells of the liver lobules with loss of nuclei and fragmentation of the cells. This change, although observed in nearly all animals in a mild form, was present to a marked degree in 54% of the dogs, and was occasionally present (11%) to the extent commonly seen following certain fatal toxemias. A much more frequent change, but with questionable significance, was a widening of the sinusoidal spaces and thinning of the hepatic cells into cords of cells which had apparently retained their vitality. Occasionally the large sinusoidal spaces intervening between these cords of cells were filled with blood. Usually, however, only a small amount of blood, or none, was present. Cloudy swelling was of course present in varying degrees. The same kind and degree of pathological change was observed in animals dying from obstruction accompanied by strangulation, except that the percentage of animals revealing the most marked damage was greater (27% as compared with 11%). Control biopsy at operation was done in each case.

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[1] Colp, R., and Louria, H. W., *Arch. Surg.*, 1925, **10**, 756.

[2] Seulberger, P., *Beit. z. Klin. Chir.*, 1930, **148**, 583.

[3] Trinchera, Carmelo, *Ann. Ital. Chir.*, 1931, **10**, 682, Abst. in *Zent. f. d. ges. Chir.*, 1932, **56**, 294.

Hepatic function tests using 40 mg. phenoltetraiodophthalein[4] per kilo of body weight were performed on 16 occasions in animals with intestinal obstruction and on 6 occasions in animals with strangulation in addition to obstruction. In 29 control dogs the average retention was 9.8% in 5 minutes and 4% in 10 minutes, which is similar to the normal figures reported previously by Graham, Cole, Copher, and Moore.[4] The average of the readings on animals with intestinal obstruction at various stages of illness was 21% after 5 minutes and 10.6% after 10 minutes. The retention in animals ill with strangulation was slightly higher, i. e., 24.5% and 12.5% for the 5 and 10 minute periods respectively. When an accidental infection such as local peritonitis or abscess was found, the retention figures were the highest. This observation agrees to a large extent with clinical data and suggests that the excretion test of hepatic function is more sensitive to infection within the liver than to any other pathological condition. It is possible that there is an absorption of bacteria from the obstructed or strangulated intestine and that the resulting infection accounts for the hepatic changes we found. However we saw bacteria in considerable numbers in the liver in only 3 of our dogs. We realize however that they may have been present in many more. Cultures were not made.

We also collected samples of lymph from the thoracic duct, portal blood and systemic blood before and after intestinal obstruction and strangulation and injected them into guinea pigs and mice intraperitoneally. Although occasionally the samples obtained after obstruction and strangulation were more toxic than before the conditions were produced, the difference was so slight that the absorption of a toxin from the obstructed or strangulated loop could not be assumed.

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[4] Graham, E. A., Cole, W. H., Copher, G. H., and Moore, S., Diseases of the Gallbladder and Bile Ducts, Lea and Febiger, Phila., 1928.

6320

Combined Effect of Ephedrin and Atropin on Complete Heart Block.

S. N. CHEER, C. L. TUNG AND C. W. BIEN.

From the Department of Medicine, Peiping Union Medical College.

The use of ephedrin in complete heart block was first reported by Miller and later by Stecher. But the combined action of ephedrin and atropin on the human heart according to Chen and Schmidt[1] has never been investigated.

A case of complete heart block without Adams-Stokes' attacks was available to study its reaction to ephedrin alone. The patient was kept quiet in bed and undisturbed. Electrocardiograms were taken before and at frequent intervals after the administration by mouth of ephedrin. Blood pressure readings were made at frequent intervals and usually before the electrocardiograms, from which both auricular and ventricular rates were obtained. Results are shown in Table I. It will be seen that after the administration of 150 mg. of ephedrin in 20 minutes, there was no increase in ventricular or auricular rate, but an increase of 5 mm. Hg. in systolic and diastolic blood pressure.

TABLE I.
Effect of Ephedrin on Complete Heart Block.
(Ephedrin 0.09 gm. at 10:30 a.m., and 0.06 gm. at 10:50 a.m., a total of 0.15 gm. in 20 minutes.)

Time. a.m.	Vent. rate per min.	Aur. rate per min.	B. P. mm. Hg.
10:28	38	91	110/70
10:40	38	82	110/70
11:00	38	80	110/75
11:20	37	80	115/75
11:50	38	79	115/75
12:10	38	78	115/75

Different explanations have been offered for the negligible pressor effect occasionally encountered as in this case. Chen and Schmidt attributed the depressant effect of large doses of ephedrin either to direct depression of heart muscle, or to a difference in the quantity of "receptors" present in the individual. Kreitmair explained the difference on the basis that small doses of ephedrin stimulate the sympathetic nerve endings, while large doses stimulate the parasympathetic. However in animal experiments according to Fujii and Kreitmair this depressant action of ephedrin on the heart is not antagonized by atropin, while on the other hand Mehes and Kokas believed that the depressant effect was partly removed by atropin. To test the validity of these hypotheses in the clinical case, atropin was given after the patient had been ephedrinized. The results are shown in Table II. It will be seen that atropin hastens and intensifies the pressor effect but shortens the duration. Such combined action of ephedrin with atropin suggests that atropin neutralizes the parasympathetic effect that results either from the individual's vagotony or from the stimulating action of ephedrin on parasympathetic nerves. If the lack of pressor effect after therapeutic doses of ephedrin is due to either limited quantity of "receptors" or depressant action of ephedrin as suggested by Chen, then atropin must increase if not in amount at least in sensitivity these "receptors" or abolish the depressant action of ephedrin on human heart. Two individuals with normal hearts were studied and found to have the same reaction as the patient.

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[1] For literature, see references of Chen & Schmidt's review of Ephedrin and Related Substances in *Medicine*, 1930, **9**, 94.

TABLE II.
Combined Effect of Ephedrin and Atropin on Complete Heart Block.

Time a.m.	Vent. rate per min.	Aur. rate per min.	B.P. mm. Hg.	Remarks
9:08	37	94	110/70	Before ephedrin
9:10				Ephedrin 0.09 gm.
9:30	37	77	110/70	
9:32				" 0.06 "
9:40	37	74	110/75	
10:00	38	69	115/80	
10:02				Atropin 0.0009 gm. hypodermically
10:05			150/100	
10:20	39	94	160/100	
10:45	40	115	160/95	
11:45	39	115	120/70	

In order to exclude a possible abnormal reaction of this patient to atropin 2 mg. of atropin alone were given hypodermically. The results showed an increase of 30 beats in auricular rate, and a decrease in ventricular rate of 2 beats per minute. Both systolic and diastolic pressure showed a fall of 10 mm. Hg. at the height of the effect. Evidently this patient reacted to atropin as usual.

6321

The Precipitin-Ring Test Applied to Fungi.*

GEORGE K. K. LINK, ADELINE DE S. LINK, GEORGE L. CROSS AND
HAZEL W. WILCOX. (Introduced by F. C. Koch.)

From the Departments of Botany and Chemistry, University of Chicago.

During a study of the phenomena of resistance and susceptibility of plants to pathic events, experiments have been made to determine whether serological techniques could be used as tools for the study of phytopathogenic microparasites, and of their parasitic and pathogenic relations to plants. In the course of experiments from 1925 to 1928 it was found that in many cases serological specificity of phytopathogenic schizomycetes as manifested in the agglutination test apparently is correlated with host, and even symptom specificity within the same host. Incidentally, Sharp made the first report of finding a smooth virulent, and a rough avirulent strain of a phytopathogenic bacterium, *Phytomonas phaseoli sojense,* which parasitizes the soy bean plant.[1]

We chose next to determine whether serological techniques could be used in the study of phytopathogenic fungi and their nonpathogenic taxonomic allies.† To put the methods to severe test we chose to begin with members of the form genus *Fusarium,* phytopathogenically the most important genus of the division *Phragmosporae* of the so-called "fungi imperfecti". According to Wollenweber[2] this genus includes 64 species, with 79 varieties and 38 forms. These have been arranged in 16 sections with 9 subsections, 12 of

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* The present investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

[1] For a summary and literature citations of most of this work and of relevant work by others with phytopathogenic bacteria, see Link, G. K. K., Chap. 44, p. 590-606, in Jordan, E. O., and Falk, I. S., "The Newer Knowledge of Bacteriology and Immunology," University of Chicago Press, Chicago, 1928. 1196.

† This work was begun by 2 of us (G. K. K. L. and A. DeS. L.) in 1927 with equipment provided us in the Department of Hygiene and Bacteriology through the courtesy of Dr. W. H. Taliaferro. In 1929 a grant was received from the Rockefeller Foundation, and since then the work has been carried on uninterruptedly with the assistance of Dr. George L. Cross (1929-1930) and of Hazel W. Wilcox (1930-—). Upon completion of the New Botany Laboratory, made possible in part by a grant to the University from the General Education Board, the work has been carried on in the Botanical Laboratories.

[2] Wollenweber, H. W., *Fusarium Monographie, Fungi parasitici et saprophytici. Z. für Parasitenkunde. Abt. F. Zeitschrift für wissenschaft. Biol.*, 1931, **3**, 19.

the sections including members for which the perfect ascomycete stage is known. This genus includes many parasites which are part of the etiological complex of interesting and economically important plant diseases. Identification and classification of the Fusaria is often difficult or impossible because when grown on artificial media they may fail to produce the characteristic structures necessary for their differentiation.

The work was begun with 2 species, *F. conglutinans,* incitant of cabbage yellows, and *F. cubense,* incitant of banana wilt. In the course of the past 5 years, as different problems of technique arose, 19 species of Fusarium have been used as inject and/or as test antigens against all the others. These are: *F. anthophilum, F. argillaceum, F. conglutinans, F. cepae, F. cubense, F. decemcellulare, F. dimerum, F. fructigenum, F. graminearum (Gibberella Saubenetii), F. javanicum, F. lycopersici, F. moniliforme, F. nivale, F. ossiculum, F. oxysporum, F. semitectum, F. sporotrichioides, F. theobromae,* and *F. trichothecioides.* In addition *F. conglutinans var. callestephi,* 2 strains of *F. cubense,* and one strain of *G. Saubenetii* have been used. *F. acuminatum, F. sambucinum,* one species each of Ramularia and of Cylindrocarpon, *C. album,* one strain of *Sclerotinia sclerotiorum,* and the + and— strains, singly and jointly of *Neurospora tetrasperma* have been used as inject and/or as test antigens, against the antiserum of one or more of the other inject antigens.

Since no serological studies of these organisms were reported until our work was in progress[3] considerable preliminary experimentation had to be done. The following problems have been investigated:

1. Search for a suitable protein-free medium revealed that Richard's solution‡ could be used for all the fungi tested. Good mats usually developed in subdued light at 20-30°C. in 3-4 weeks.
2. A method has been developed for recovering the fungus mats from these cultures, drying them with a minimum of autolysis, and rapidly preparing fungus powders which pass a 100 mesh sieve.
3. Studies were made to determine the best treatment of powders to obtain saline extracts with maximum antigen content.

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[3] Coons, G. H., and Strong, M. C., Mich. Acad. of Sciences, Arts and Letters, 1929, 9, 65.

‡	gm.		gm.
Sucrose	33.3	KH_2PO_4	3.3
KNO_3	6.6	$MgSO_4$	1.7

H_2O (distilled) 1000.0 cc.

4. Experiments were made to determine the relative merits of agglutination, precipitin, and precipitin-ring tests. The last was found to be the most feasible.

5. Micro-Kjeldahl analyses were used as a basis for calculation of the amounts of protein in each powder and saline extract in an attempt to correlate the amount of protein in the inject and test antigens and the titres obtained in the serological tests.

6. The relative merits of intravenous and/or intraperitoneal injection of rabbits were determined. Intravenous injection of the clear saline extracts of the powders and intraperitoneal injection of saline re-suspensions of the extracted residues were found best.

7. For the production of most specific titres the optimal dosages, intervals of injection, and bleeding after sensitization were determined.

8. The effect upon the quality and titre of the antisera of age, moulting, and temperature of environment of the rabbits, as well as the effect of temperature and time upon the antisera *in vitro* were determined.

9. Studies were made to determine the best method of preparing test antigens suitable for the precipitin-ring test. Grinding of the powders with ligroin-extracted pumice and their subsequent extraction with petrol ether (ligroin, B.P. 30-60°C. Max. B. P. 80°) preliminary to extraction at 0°C. with saline for 18 hours, followed by centrifugation and filtration through coarse filter paper, were found adequate to yield clear, potent test-antigens.

10. Studies were made of the layered tubes with respect to the incubation temperature and reading intervals most conducive to detection of clear rings and maximum titres.

11. Tests were made to determine the relative efficacy of undiluted antisera, and of antisera diluted with glycerine, glucose, and hypertonic salt solution, when tested against progressive dilutions of the test antigens. The undiluted antisera were most satisfactory.

12. Test antigens were prepared with buffered saline solutions to determine the effect of pH upon the range and specificity of the reactions.

13. Absorption tests were made to differentiate between closely related antigens.

14. A study of skin tests has been begun to further investigate correlation of serological and morphological relationship of the fungi.

All fungi tested are antigenic as determined by the precipitin-ring test. Titres up to 1:25,600 have been obtained.

All but one of the animals tested (120) produced antisera. The normal sera of very few (2) gave feeble reactions above the lower dilutions (1:200) of the test antigens.§

6322

Influence of Acute Infection and of Artificial Fever on Plasma Lipoids.

IRVINE MCQUARRIE and A. V. STOESSER.

From the Department of Pediatrics, University of Minnesota Medical School.

During another investigation on the lipoids of the plasma in children, it was observed in several instances that values for lecithin, cholesterol and total fatty acids were all markedly influenced by acute infections. Although the literature revealed the fact that cholesterol has previously been found to be lowered in certain acute infectious diseases[1, 2] and in tuberculosis,[3, 4] little is actually known regarding the significance of this change and practically no information is available regarding the effect of infection on the lecithin and total fatty acid levels in the blood.

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§ We are indebted to Dr. John R. Johnston of the United Fruit Company, and to Dr. W. H. Taliaferro of the Department of Hygiene and Bacteriology, for aid in obtaining various strains of *Fusarium cubense;* to Dr. Alice A. Bailey of the Cooperative Laboratory of the Office of Horticultural Crops, United States Department of Agriculture, and of the Botany Department of the University of Chicago, for most cultures of the fungi used and for helpful suggestions; to Dr. C. D. Sherbakoff of the Tennessee Agricultural Experiment Station for cultures of Ramularia and Cylindrocarpon; to Dr. H. H. Whetzel of Cornell University for a culture of *Sclerotinia sclerotiorum;* to Dr. B. O. Dodge of the New York Botanic Garden for cultures of *Neurospora tetrasperma;* to Dr. A. S. Dahl of the Green Section of the United States Golf Association for a culture of *F. nivale;* to Miss Helen Johann of the Office of Cereal Crops, United States Department of Agriculture, for cultures of *Gibberella Saubenetii.*

We are also indebted to Dr. W. H. Taliaferro for use of space and equipment in the Department of Hygiene and Bacteriology and for helpful suggestions, and to Dr. F. C. Koch for use of space and equipment in the Department of Physiological Chemistry and for helpful suggestions relative to preparation of powders and to micro-kjeldahl determinations of nitrogen.

Finally we are grateful to the Rockefeller Foundation for the grant which made continuation and enlargement of this research possible, and to the Committee of the Biological Division of the University of Chicago, which recommended this research for a grant.

[1] Denis, W., *J. Biol. Chem.*, 1917, **29**, 93.

[2] Kipp, H. A., *J. Biol. Chem.*, 1920, **44**, 215.

[3] Eichelberger, L., and McCluskey, K. L., *Arch. Int. Med.*, 1927, **40**, 831.

[4] Henning, B. H., *J. Biol. Chem.*, 1922, **54**, 167.

The various lipoid fractions have been determined by the methods of Bloor[5, 6, 7] in 15 children with various acute infections, first at the height of the disease and again after recovery. To ascertain the influence of fever *per se* as against that of the other effects of infection, determinations were made before, during and after the artificial production of fever, in one case of *dystonia progressiva deformans* by use of phenylethylhydantoin and in one case of chronic arthritis by diathermy. Determinations were also made before, during and after high fever on 2 occasions in each of 3 congenitally syphilitic children, who were inoculated with plasmodia malariae as a therapeutic measure. No particular attention was given to the factor of diet in the first 7 cases with acute infection, but in the other 13 cases studied a standard diet was given several days before and throughout the entire period of observation. The diet contained adequate amounts of vitamins and water and 2 gm. each of protein and fat and 5 gm. of carbohydrate per kilo of body weight. The 15 cases with acute infection were distributed as follows: Uncomplicated pneumonia, 6; pneumonia complicated by mastoiditis, otitis media or empyema, 3; uncomplicated acute tonsillitis and nasopharyngitis, 3, and one each of acute appendicitis, acute otitis media and acute rheumatic fever.

The results may be briefly summarized as follows: In every case of acute infection studied there was at the height of the disease a definite lowering of cholesterol, lecithin and total fatty acid. In all but one case the values became definitely subnormal but rose again to normal during convalescence. There appears to have been no constant relationship between the height of the fever and the degree of hypocholesterinemia or hypolecithinemia in these cases. Diet had no demonstrable influence on the results. The average value for the cholesterol at the height of the infection was 120 with variations between 61 and 175 mg. per 100 cc. of plasma. Corresponding figures for the lecithin at the time of the infection were, average 98, low 60, and high 150 mg. per 100 cc. The average value for cholesterol after recovery was 196 mg., while that of lecithin was 170 mg. per 100 cc. of plasma. Changes in the total fatty acid values paralleled those for the cholesterol and lecithin fairly closely

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[5] Bloor, W. R., *J. Biol. Chem.*, 1916, **24**, 227.

[6] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

[7] Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

in most instances. The total leucocyte count tended to vary inversely with the lipoid content of the plasma.

The 3 cases of congenital syphilis showed definite hypocholesterinemia during the course of malarial infection but there was no significant difference between the values obtained at the height of the paroxysm and those found during the afebrile intervals. But one sample of plasma was taken after disappearance of the malaria under quinine therapy. This showed a rise in cholesterol from 85 to 170 mg. per 100 cc. The lecithin and total fatty acids remained within normal limits at comparatively constant levels in these 3 cases. That elevated body temperature *per se* is not responsible for the lowering of the various lipoid constituents is indicated by the fact that fever artificially produced by phenylethylhydantoin failed to have any such effect. The values in the 2 subjects examined were in the upper reaches of the normal zone before the hyperthermia was produced and remained at the same or at slightly higher levels during and after the febrile periods.

6323

Effect of Sprint Running on the pH of Venous Blood.

E. P. LAUG, J. A. NAVE AND N. D. PERSON.
(Introduced by H. C. Bazett.)

From the Department of Physiology, University of Pennsylvania Medical School.

Severe exercise which taxes the human organism to its limits has been shown to produce very profound changes in the blood. In these preliminary experiments, venous blood from 3 runners was obtained. As soon as possible after the run, blood from arm veins was drawn without CO_2 loss into syringes containing carefully neutralized potassium oxalate. The syringes were iced and immediately transported to the laboratory where the pH of the whole blood was determined at 38° C. with a glass electrode. The exercise consisted of running 440 and 880 yds. without competition. The runners were in different states of training, but the small number of determinations does not justify an attempt to correlate change in blood pH and muscular ability. The chief interest is that these pH values of blood after such severe exercise are probably some of the most acid ever obtained in normal subjects.

Great difficulties were encountered in drawing blood samples immediately after the exercise, due to almost total absence of skin blood and collapse of the superficial veins. In order to facilitate collection of blood, stasis was applied in several instances. But control experiments indicated that such a procedure must be avoided if correct results are to be obtained. However, stasis for 45 sec. during resting conditions, does not seem to induce any very marked effects.

It is also to be noted that there appears a rough correlation between the severity of the run as determined by the time performance and the degree to which the pH is depressed. The return to normal pH is in general comparatively slow, at least within the first 5 minutes after the exercise.

The following is example of 1 series of tests:

TABLE I.

Date	Run	Timed		Sample obtained after end of exercise		Stasis applied		pH
				Time		Time		
1932	Yds.	Min.	Sec.	Min.	Sec.	Min.	Sec.	
Subject, J. A. N.								
Apr. 25	440	1	3	1	15	1	15	7.15
May 3	440		59		30	No stasis		7.15
				4	6	"		7.19
" 10	440		58		50		30	7.13
				2	30	1		7.02
				4	45		30	7.00
" 17	440		57	1			30	7.16
				2	20	1	7	7.07
				5	12		30	7.11
" 20	Normal	Resting				No stasis		7.38
	"	"					45	7.38
Subject, M. D. P.								
Apr. 25	440	1		1	15	1	15	6.95
Apr. 26	440		54	1	40	No stasis		7.04
May 3	440		59	2	26	"		7.20
May 13	440	1			40	"		7.14
				1	50		45	7.13
May 19	880	2	6	1	5	No stasis		6.98
				2	5	1	20	6.85
				5	25	No stasis		6.98
May 20	Normal	Resting				No stasis		7.39
Subject, W.								
May 5	440		58		30		30	6.95
				1	5	No stasis		7.22

6324

Calcium and Nitrogen Content in Urine of Normal and Cancer Mice.*

I. A. PARFENTJEV.

From the Department of Pathology, Washington University School of Medicine, St. Louis.

Crocker mouse sarcoma 180 represents a very stable type of tumor. After inoculation into the strain of mice used by us the tumor grows in 95-98% of the animals. Its rate of growth is very rapid. The animals usually die about 6 weeks after transplantation.

We observed that the composition of the urine of the sarcoma mice differs in certain respects from that of the urine of normal mice. The urine of normal mice gives a copious precipitate on addition of metaphosphoric, acetic acid (Bang's reagent) or sulphosalicylic acid; this precipitate is markedly diminished in the urine of mice previously inoculated with sarcoma 180. Before the addition of the precipitating reagent, the urine was filtered or centrifuged and the supernatant fluid decanted. The urine of mice has a pH 5.6. After filtration the urine still remains opalescent. Following addition of a diluted mineral acid the urine becomes turbid.

We observed that the addition of sodium fluoride preceding the addition of the precipitating reagent inhibits the formation of the precipitate in the urine of normal mice which otherwise takes place under the influence of metaphosphoric acid. This indicated the importance of calcium in the production of the precipitate.

We compared therefore the concentration of calcium in urine of 150 normal and 150 sarcoma mice. Amount of Ca in mg. per 1 cc. of urine: Normal mice, 0.056-0.13; cancer mice, 0.023-0.042.

Urine of cancer mice contains therefore a smaller amount of calcium than urine of normal mice. It was possible to increase the amount of calcium in urine, normal as well as of cancer mice, by the previous oral administration of calcium salts to the animals. Thus after oral administration of 0.1 gm. of calcium gluconate in 2 cc. H_2O we found in 1 cc. of urine of a sarcoma mouse collected during the following 12 hours 0.5 mg. Ca. After oral administration of 0.025 gm. of calcium gluconate in 2 cc. H_2O, 1 cc. of urine collected during the following 12 hours contained 0.18 mg. Ca.

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*These investigations were carried out partly with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation. I wish to express my thanks to Dr. Leo Loeb for his suggestions during the course of these experiments.

The precipitate obtained from the urine of mice by metaphosphoric or acetic acid is insoluble in cold water, in 70% alcohol and in dilute acids, but it is readily dissolved in weak and strong alkali as well as in strong mineral acids. The precipitate dissolved in weak alkali could easily be reprecipitated by addition of weak acids. The fact that such a precipitate could be obtained from urine of mice by means of metaphosphoric acid, acetic acid, or sulphosalicylic acid, and furthermore the positive Heller's ring test with urine, as modified by Roberts, indicates the presence of protein in the urine of mice.

The biuret test with the precipitate is likewise positive.

The *total nitrogen* content of urine of mice amounts to 25-30 mg. of N per 1 cc. of urine. We then made a separate analysis of nitrogen content of the precipitate and of the supernatant portion of the urine in which precipitate had been produced with acetic acid (Bang's reagent). From the data obtained it was possible to calculate the amount of coagulable and noncoagulable nitrogen in the total urine.

Amount of nitrogen in mg. in 1 cc. of urine.

	Coagulable N (corresponding to N of precipitate)	Noncoagulable N.
Normal mice	5.6-10	20-24
Cancer mice	2 - 3	19-25

These results indicate that after transplantation of sarcoma 180 there takes place in the urine of tumor mice (1) a diminution in the concentration of calcium; (2) a diminution in the amount of coagulable nitrogen.

6325

Ineffectiveness of Calcium, Potassium, and Magnesium Salts Against Symptoms of Anaphylactic Shock.

SAMUEL H. HURWITZ AND ARTHUR L. WESSELS.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine.

It is well known that changes in the balance of calcium and potassium ions may produce definite effects upon the neurocellular responses of animals and man. Warnant,[1] and Thornton and Gillespie[2] have shown that such alterations in the concentration of these ions may influence the bronchiolar reactions of the isolated perfused lungs of animals; addition of calcium to or a reduction of potassium in the perfusion fluid resulting in bronchoconstriction. With magnesium alone Warnant obtained bronchodilation. In intact animals, Anderson and Rosenau[3] showed that magnesium did not in any way affect the fatal outcome of anaphylactic shock.

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[1] Warnant, H., *Arch. Internat. de Pharm. et de Therap.*, 1930, **27**, 61.

[2] Thornton and Gillespie, cited by Bray, G. W., Recent Advances in Allergy. Blakeston's. 1931. p. 27.

The experiments here recorded were undertaken to obtain further knowledge concerning the value of calcium, potassium, and magnesium salts as protective agents against the symptoms of anaphylactic shock. Thirty-three sensitized guinea pigs were used: 15 animals were injected with calcium lactate (0.7 to 1.5 gm. per kilo), 9 with potassium chloride (20 to 30 mg. per kilo.) and 9 with magnesium sulphate (0.7 to 1.0 gm. per kilo). The salts were administered intramuscularly and the dosage so chosen that a maximum effect would result without masking the symptoms of shock. The interval of time between the injection of the salt and the critical dose of horse serum varied from 20 to 40 minutes. That the doses selected were large was shown by the death of a number of unsensitized control animals within 24 to 48 hours after the salts were given. Magnesium sulphate usually produced marked muscular relaxation and some anesthetic effect; calcium and potassium however, gave rise to no immediately discernible clinical symptoms.

The technic used for sensitization and production of shock was the same as that recorded in previous studies: the animals were sensitized by the intraperitoneal injection of 1 cc. horse serum, and shocked after an incubation period of 2 to 3 weeks by the intracardiac injection of 0.5 cc. serum. Control observations of this procedure carried out in connection with other experiments[4] showed a mortality of about 85%. Compared with this control mortality, the animals given preliminary injections of the calcium, potassium, and magnesium salts showed about the same mortality rate. Of the guinea pigs given calcium lactate 80% died; in the potassium chloride series 88% succumbed; and all animals given magnesium sulphate died in anaphylactic shock.

It would appear, therefore, that changes in the cellular concentrations of calcium, potassium, and magnesium ions produce no effect on the fatal outcome of anaphylactic shock. Injections of magnesium sulphate, moreover, resulted in a higher mortality (100%) despite its central depressant action. In this respect it differs markedly from phenobarbital, whose central depressant action we have found[4] to reduce the mortality from anaphylactic shock by over 50%.

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[3] Anderson, J. F., and Rosenau, M. J., *J. Med. Res.*, 1909, **21**, N. S. 16, 1.

[4] Hurwitz, S. H., and Wessels, A. L., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 1016.

6326

Intranuclear Inclusions in Pertussis.

HAROLD ANDERSON MC CORDOCK.

From the Department of Pathology, Washington University School of Medicine.

For many years pathologists have been interested in a peculiar type of pneumonia that frequently complicates certain epidemic diseases, termed interstitial bronchopneumonia because of the cellular infiltration in the walls of the bronchioles and alveoli, and commonly seen in epidemic influenza, measles and pertussis. Dr. Muckenfuss and I[1] have been impressed by the similarity of the pneumonia produced by vaccine virus in the rabbit's lung to human interstitial pneumonia. We, therefore, suspected that a virus might be concerned with human interstitial pneumonia in measles, influenza and whooping cough. Measles is a virus disease, and many investigators now believe that the primary etiological agent of epidemic influenza may also be a virus. On the other hand, the Bordet-Gengou bacillus is almost universally regarded as the etiological agent of pertussis; although a critical study of the relation of this bacterium to the disease reveals many discrepancies. (Löwenthal and St. Zurukzoglu.[2]) Furthermore, a vaccine prepared from the bacillus is of doubtful value both as a prophylactic and as a curative agent. Pertussis sometimes is complicated by encephalitis, as are the known virus diseases such as measles and vaccinia. Finally, the interstitial character of the pneumonia associated with pertussis is similar to that seen in other virus diseases and therefore suggests the possibility of a virus in pertussis also.

Since inclusion bodies are associated with many virus diseases, we examined the sections from 35 cases of pertussis and found intranuclear inclusions in the lungs of 12. These were found in the cells lining the alveoli as well as in the bronchial epithelium. The cells containing the inclusions were always larger than their neighbors and their cytoplasm more basophilic. In a few cases, they were found in liver cells about areas of necrosis and, under the same circumstances, in cells of the adrenal. They have been observed also in the mucous glands of the smaller bronchi. These inclusions are acidophilic, and a clear zone separates the inclusion from the nuclear membrane to which small fragments of chromatin are often found adhering.

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[1] Muckenfuss, R. S., McCordock, H. A., and Harter, J. S., *Am. J. Path.*, 1932, **8**, 63.

[2] Löwenthal, W., and St. Zurukzoglu, *Kolle u. Wassermann*, 1928, **5**, 1271.

As control material we examined the organs from 90 autopsies, in none of which pertussis had been diagnosed. Of these, 78 were children dying of various diseases, including measles, diphtheria and epidemic influenza, and 12 were adults who succumbed to influenza during the 1918-19 pandemic. In the lungs of 2 of the infants, 4 and 5 months old, similar inclusions were found. In each instance there was present also a peribronchial infiltration with large mononuclear cells and lymphocytes.

The presence of these inclusion bodies in slightly more than one-third of all the cases of pertussis and their absence in all but 2 of the 90 control cases seems significant.

Intranuclear inclusions not unlike those described here in association with pertussis have been found in various organs by a number of investigators. Jesionel and Kiolemenoglou[3] first reported them in the lungs, liver and kidneys of a premature syphilitic foetus. Farber and Wolbach,[4] reviewing the literature, tabulate 8 reported cases in which such inclusions were found in the lungs and add 2 more of their own. Von Glahn and Pappenheimer[5] report inclusions in the organs of a man 36 years old, but all the other reported instances noted in the lungs, concerned either stillborn foetuses or infants under 2 months of age. Pneumonia or interstitial cellular infiltration was reported in only 2 cases, namely those of Smith and Weidman[6] and of Goodpasture and Talbot.[7] In none of these 10 cases in which inclusions occurred in the lungs is there any mention of pertussis. However, Feyrter[8] reported intranuclear inclusions in the lungs of an infant 2½ years of age, whose fatal illness had been pertussis. Our cases of pertussis in which inclusions were present in the lungs outnumber all the other reported cases in which they have been observed in that organ.

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[3] Jesionel and Kiolemenoglou, *München. med. Wehnschr.*, 1904, **43**, 1905.

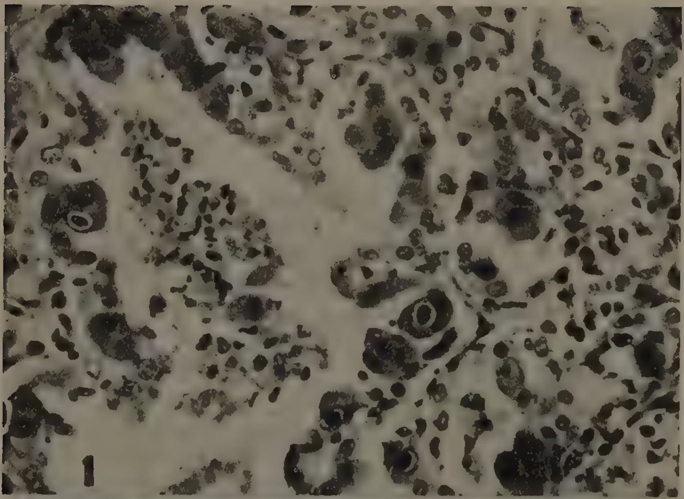
[4] Farber, S., and Wolbach, S. B., *Am. J. Path.*, 1932, **8**, 123.

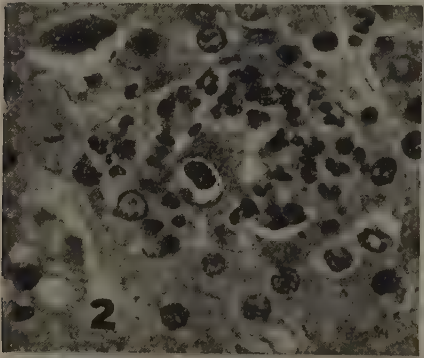
[5] Von Glahn, W. C., and Pappenheimer, A. M., *Am. J. Path.*, 1925, **1**, 445.

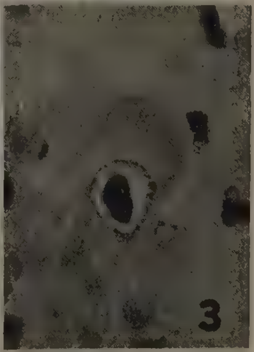
[6] Smith, A. J., and Weidman, F. D., *Am. J. Trop. Dis.*, 1914, **2**, 256.

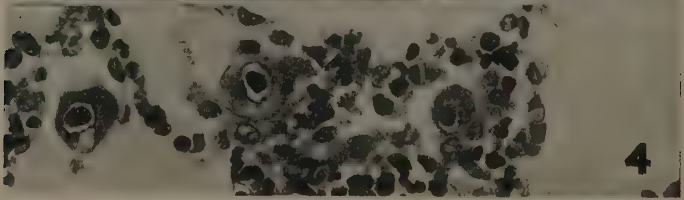
[7] Goodpasture, E. W., and Talbot, F. B., *Am. J. Dis. Child.*, 1921, **21**, 415.

[8] Feyrter, F., *Frank. Z. f. Path.*, 1927, **35**, 213.









1. Lung with several intranuclear inclusions in the cells lining the alveoli.
2. Focal necrosis of liver with inclusion in liver cell.
3. Oil immersion micrograph of an inclusion shown in Fig. 1.
4. Lung, showing 3 inclusions. One cell is free in the alveolus.

It is impossible to decide in what way, if at all, the intranuclear inclusions we have found so frequently in pertussis are related to those already described in a variety of pathological conditions. Inclusions have not yet been observed in the salivary glands from cases of pertussis, but in only 3 instances have these organs been available for examination.

The presence of intranuclear inclusions in the lungs in such a high percentage of our cases of pertussis and their infrequency in a variety of other childhood diseases indicate that they are intimately associated with whooping cough and suggest that the possible rôle of a filterable virus must be considered in this disease.

6327

Note on the Cultivation of the Typhus Fever Rickettsia.

CLARA NIGG and K. LANDSTEINER.

From the Laboratories of the Rockefeller Institute for Medical Research.

In our recent paper[1] on the cultivation of the typhus fever rickettsiae, it was stated that "of several tissues tested, only tunica and peritoneum gave satisfactory results in the medium described." Since then, we have succeeded in obtaining good growth with guinea pig testicle and kidney tissues in the serum Tyrode medium. Multiplication also occurred when various other tissues (guinea pig brain, heart, adrenal, lung, spleen, embryo) were used.

In these experiments, phenol red was added to the Tyrode solution in order to follow changes in reaction, as produced by the tissues during the incubation of the cultures. By reducing the amount of tissue in the medium, it was possible to retard the change in reaction, a circumstance which in our opinion may be significant in obtaining positive results.

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[1] Nigg, C., and Landsteiner, K., *J. Exp. Med.*, 1932, **55**, 563.

6328

Paradoxical Increase of Phosphatase Activity in Preserved Serum.

AARON BODANSKY. (With the assistance of Lois Hallman.)
From the Laboratory Division of the Hospital for Joint Diseases, New Yorks City.

In the course of examination of the liability of serum phosphatase several samples of serum were saved for 24 hours under ordinary laboratory conditions (for about one-half to 1 hour at room temperature and in the refrigerator thereafter). It was expected that the phosphatase values would be similar to or lower than the values found in fresh serum. The following data, typical of results obtained in about 30 different comparisons, will show unexpectedly increased values.

In the course of examination of the lability of serum phosphatase

TABLE I.
Inorganic phosphorus is stated in mg., and phosphatase in units per 100 cc. of serum.

Sample No.	Inorganic P Fresh	Inorganic P 24 hrs.	Phosphatase Fresh	Phosphatase 24 hrs.	% Inc.
210	8.6	8.7	8.8	10.5	19.3
222	8.2	8.1	5.0	5.8	16.0
223	9.3	9.4	6.5	7.0	7.6
213	7.3	....	13.6	16.4	20.5
Ji	2.5	....	49.0	52.5	7.2
Jo	5.2	....	10.2	12.5	23.0

In these tests the differences between the 24-hour serum and the fresh serum phosphatase activities were greater than discrepancies usually found in determinations performed simultaneously upon 2 samples of the same serum (about 2%, rarely as high as 5%), and they were all *in the same direction.* It may be noted that the inorganic phosphorus in the preserved serum remains unchanged.

The interest of these results lies in their unexpectedness. Under the conditions of the test[1, 2] the assumption of an increased pH causing increased phosphatase activity is excluded (1) because our buffer maintains a pH constant during hydrolysis at about 8.7 and (2) because any slight increase in the pH that might occur with the preserved serum could not cause increases of phosphatase activity as great as those observed. It is possible (1) that during the 24 hours intervening between the separation of the serum and the analysis some factor inhibiting phosphatase activity is destroyed; (2) that a component of serum phosphatase is activated; or (3)

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[1] Bodansky, A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 760.

[2] Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 199.

that substances are formed which further phosphatase activity. However, the mechanism and significance of this apparent increase in phosphatase activity of preserved serum remain to be elucidated.

6329

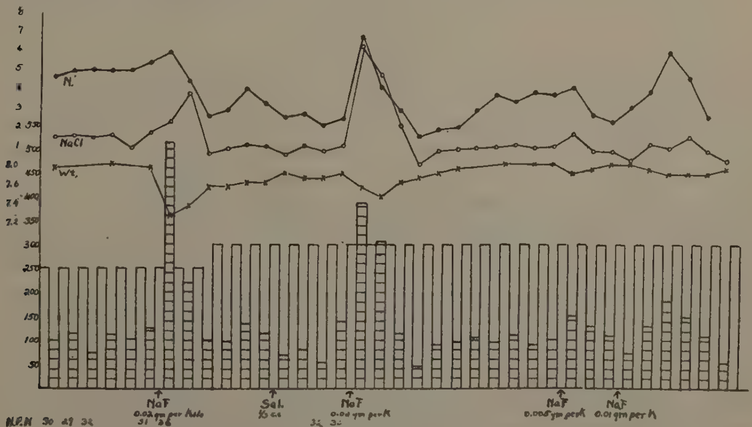
Diuretic Action of Sodium Fluoride.

LEO GOTTLIEB and SAMUEL B. GRANT (Introduced by D. P. Barr).

From the Department of Medicine, Washington University, St. Louis.

During a study of the effect of sodium fluoride on the thyroid gland of dogs, we noted that the animals had a pronounced increase of urine output after intravenous injection of the drug. Goldenberg had similarly noted a diuresis in goiter patients treated with sodium fluoride[1]. The present study was undertaken to further investigate this diuretic action.

Dogs were placed in metabolism cages and given a weighed diet of Purina Dog Chow to fulfill the food requirements and to provide a constant ingestion of salt and protein. Water was given in sufficient, but constant, daily amounts. After a control period, during which the body weight, urine output, urine chlorides and total urine nitrogen were determined daily, sodium fluoride in 1% aqueous solution was injected intravenously. The effect is shown in the following charts. The experiment was repeated and confirmed in six different animals.



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[1] Goldenberg, Leon, *Presse medicale*, 1930, **38**, 1751.

Single doses of 0.005 gm. to 0.020 gm. per kilo of body weight were followed by a pronounced diuresis. In addition to an increased water output, the urine chlorides and nitrogen were strikingly increased. The control urine was acid, but, after sodium fluoride, it was strongly alkaline to litmus and this alkalinity persisted for approximately one week after the injection. The non-protein nitrogen of the blood has not been fully studied, but in one animal the only change noted was an increase from an average of 31 mg.% to 36 mg. % during the diuresis. This is possibly due to dehydration and blood concentration. Examination of the urine, including a catheterized specimen, revealed no albumin, sugar, casts, leukocytes or erythrocytes. The kidneys of dogs that had received varying doses of NaF, including one animal that had been given 0.02 gm. per kilo of body weight daily for 41 days, showed no microscopic evidence of damage to either glomeruli or tubules. In the animal that had received daily doses for almost 6 weeks, the glomeruli were mildly hyperemic.

6330

Iodine Content of Certain Pathological Bloods in a Goiterous Region.

R. G. TURNER. (Assisted by Mina Z. Weeks.)

From the Department of Medical Research, Detroit College of Medicine and Surgery.

The conflicting findings of previous investigators as to what actually constitutes a normal iodine concentration in the blood and the various opinions regarding pathological iodine led us to study the iodine in diabetic patients, those suffering from thyroid disease, and of a number of pathological cases not directly associated with the endocrine system. Diabetic cases were considered because of the close similarity of this disease, experimentally and clinically, to hyperthyroidism.

The results of iodine estimation on 101 pathological bloods are presented as estimated by the author's method[1]. The investigation includes determinations on 13 diabetic in patients, 54 diabetic out patients, 15 hyperthyroid cases, 4 hypothyroid cases, and 15 miscellaneous cases. Of the diabetic in patients 46.1% are within the normal range, averaging 10.7 γ %, 23.1% are subnormal, averaging 7.5 γ %, and 30.8% are above normal, with an average of 29.2 γ %. Of the diabetic out patients 25.9% show values in the normal range, averaging 11.3 γ %, 46.3% below the normal range, averaging 6.3 γ %, and 27.8% above normal, averaging 60 γ %. In the hyperthyroid cases 33.3% fall within the normal range with an average of 11.5 γ % and 66.7% of the cases show a supernormal value, averaging 86.8 γ %. Of the 4 hypothyroid cases all gave figures within the normal range with an average of 10 γ %. In the miscellaneous pathological cases, 60% have a normal blood iodine concentration, averaging 11.6 γ %, 26.7% are below normal, averaging 6.1 γ %, and 13.3 % above normal, averaging 66.8 γ %. Of the 101 cases studied 53.1% are within the normal range with an average figure of 11.0 γ %, 32% are subnormal, averaging 6.7 γ %, and 34.6% above the normal range, averaging 60.7 γ %. All normal or non-pathological cases were within the normal range, averaging 11.0 γ %.

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[1] Turner, R. G., *J. Biol. Chem.*, 1930, **88**, 497.

A number of investigators have described cases of exophthalmic goiter and diabetes mellitus in the same patient. A review of the literature, however, convinces one that this combination is not common. Joslin[2] observed that exophthalmic goiter preceded diabetes in 5 out of 28 cases. In his series he found that hyperthyroidism preceded the diabetes more commonly when it was primary (exophthalmic goiter) than when it was secondary (toxic adenoma). Lawrence[3] and Thomson[4] treated cases of acute Grave's disease with insulin and noted a marked improvement, the thyroid decreased in size and other clinical symptoms disappeared. Sturm[5] showed that the thyroid gland liberates iodine into the arterial blood intermittently and that sympathetic stimulation increases the circulation. Logically one would assume that this is what occurs in diabetes when high iodine values are encountered. The opinion is expressed that the subnormal values found are not considered pathological but probably result from a change in the iodine content of the diet. The diabetic out patients as well as in patients are placed on special diets. A study has not been made of the iodine content of these diets as compared to the average diet of a normal individual. Scheringer[6] found the iodine content of the blood of normal persons receiving iodine-poor diets to average 8 γ %. Our findings show an average of 6.7 γ % for all pathological cases studied that showed a blood iodine concentration of less than our normal finding, 9 to 15 γ %. The subnormal values found are believed to be due to lowered exogenous iodine.

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[2] Joslin, E. P., "The Treatment of Diabetes Mellitus," 4th edition. Lea and Febiger, Philadelphia. 1928. 878.

[3] Lawrence, R. D., *Brit. Med. J.*, 1924, **2**, 753.

[4] Thomson, A. P., *Brit. Med. J.*, 1924, **2**, 876.

[5] Sturm, A., *Deutsch. Arch. klin. Med.*, 1928, **161**, 129.

[6] Scheringer, W., *Deutsch. Med. Woch.*, 1931, **22**, 883.

In hypothyroid or hyperthyroid cases the patients are not placed on special diets for long periods of time. None of the 19 cases examined showed a subnormal iodine content of the blood. Of the goiter cases only 33% were within the normal range, the remainder showing levels greater than 9 to 15 γ %. The findings show that in exophthalmic goiter cases the blood iodine content ranges 2 to 3 times the normal, in toxic adenoma with hyperthyroidism it may range as high as 5 to 20 times the normal, in simple colloid goiter the iodine content is normal, while in hyperthyroidism not clinically showing protrusion of the eye balls the iodine content may be normal, or above normal, depending on the toxicity of the goiter. No relationship between the iodine content of the blood and the basal metabolic rate could be found.

From the results presented it is believed that the blood iodine test may be of value in clinical diagnostic work, especially in differentiating between mild hyperthyroidism, exophthalmic goiter, and toxic adenoma. High blood iodine values in diabetic cases indicate hyperthyroidism accompanying or preceding the onset of clinical diabetic symptoms.

SECRETARY'S ANNUAL REPORT

April 1, 1931 to April 1, 1932

MEMBERSHIP

Members, March 31, 1931	1053	
Elected during year	91	
Honorary Members	10	
Total		1154
Resignations, year ending March 31, 1932	10	
Deaths, year ending March 31, 1932	2	
Arrears, year ending March 31, 1932	3	
Elected to Honorary Membership	1	
		16
Net Membership, March 31, 1932		1138

	1924	1925	1926	1927	1928	1929	1930	1931	1932
Membership	627	692	779	851	936	986	1026	1062	1138

SUBSCRIPTIONS

Subscriptions, March 31, 1931	397	
Subscriptions, March 31, 1932		408
Total No. of PROCEEDINGS distributed 3/31/31		1477
Total No. of PROCEEDINGS distributed 3/31/32		1562

OFFICERS elected April 20, 1932

President: A. R. Dochez.
Vice-President: E. L. Opie.
Secretary-Treasurer: A. J. Goldforb.
Council: W. B. Cannon
F. R. Sabin.

A. E. Cohn
W. J. MacNeal
W. J. V. Osterhout
W. W. Palmer
hold over

Past Presidents (By constitution): E. B. Wilson, Simon Flexner, F. S. Lee, T. H. Morgan, James Ewing, Graham Lusk, W. J. Gies, Gary Calkins, G. B. Wallace, J. W. Jobling, S. R. Benedict, Peyton Rous, Dayton J. Edwards.
Chairmen of Sections (See Sections).

Nominating Committee (elected April 20, 1932):
From New York: C. B. Davenport, R. Chambers, G. Lusk, A. F. Hess.
From Other Sections: T. Addis, A. N. Richards, R. G. Harrison, W. R. Bloor, A. C. Ivy.

Editorial Committee (appointed by Council): S. Bayne-Jones, A. J. Carlson, M. H. Jacobs, H. B. Lewis, A. B. Luckhardt, W. deB. MacNider, J. B. Murlin, A. M. Pappenheimer, H. B. Williams, A. J. Goldforb.

Membership Committee (appointed by Sections): W. R. Bloor, L. J. Cole, E. A. Doisy, R. A. Gortner, I. Greenwald, A. C. Ivy, H. Laurens, E. G. Martin, G. H. Miller.

Financial Committee: W. J. Gies, W. H. Park, A. J. Goldforb.

PAST OFFICERS

Date	*President*	*Vice-President*	*Secretary*	*Treasurer*
1903-04	S. J. Meltzer	W. H. Park	W. J. Gies	G. N. Calkins
1904-05	S. J. Meltzer	J. Ewing	W. J. Gies	G. N. Calkins
1905-06	E. B. Wilson	E. K. Dunham	W. J. Gies	G. N. Calkins
1906-07	S. Flexner	E. K. Dunham	W. J. Gies	G. N. Calkins
1907-08	S. Flexner	T. H. Morgan	W. J. Gies	G. N. Calkins
1908-09	F. S. Lee	T. H. Morgan	W. J. Gies	G. Lusk
1909-10	F. S. Lee	W. J. Gies	E. L. Opie	G. Lusk
1910-11	T. H. Morgan	W. J. Gies	E. L. Opie	G. Lusk
1911-12	T. H. Morgan	P. A. Levene	G. B. Wallace	G. Lusk
1912-13	J. Ewing	P. A. Levene	G. B. Wallace	C. Norris
1913-14	J. Ewing	C. W. Field	H. C. Jackson	C. Norris
1914-15	G. Lusk	W. J. Gies	H. C. Jackson	J. R. Murlin

			Secretary-Treasurer
1915-16	G. Lusk	G. N. Calkins	H. C. Jackson
1916-17	J. Loeb	W. J. Gies	H. C. Jackson
1917-18	W. J. Gies	J. Auer	H. C. Jackson
1918-19	W. J. Gies	J. Auer	H. C. Jackson
1919-20	G. N. Calkins	G. B. Wallace	H. C. Jackson
1920-21	G. N. Calkins	G. B. Wallace	H. C. Jackson
1921-22	G. B. Wallace	J. W. Jobling	H. C. Jackson
1922-23	G. B. Wallace	J. W. Jobling	H. C. Jackson
1923-24	H. C. Jackson	J. W. Jobling	V. C. Myers
1924-25	H. C. Jackson	J. W. Jobling	A. J. Goldforb
1925-26	J. W. Jobling	S. R. Benedict	A. J. Goldforb
1926-27	J. W. Jobling	S. R. Benedict	A. J. Goldforb
1927-28	S. R. Benedict	P. Rous	A. J. Goldforb
1928-29	S. R. Benedict	P. Rous	A. J. Goldforb
1929-30	P. Rous	D. Marine	A. J. Goldforb
1930-31	P. Rous	D. J. Edwards	A. J. Goldforb
1931-32	D. J. Edwards	A. R. Dochez	A. J. Goldforb

SECTIONS

Cleveland (Organization approved April, 1932)

Chairman: R. W. Scott. Secretary: N. C. Wetzel. Members: 27.
Meetings: Western Reserve University, May 13, 1932.

Illinois

Chairman: S. W. Ranson. Secretary: L. Arnold. Members: 95.
Meetings: Billings Hospital, November 3, 1931.
University of Illinois, December 8, 1931.
Northwestern University Medical School, January 12, 1932.
Billings Hospital, February 16, 1932.
University of Illinois, April 5, 1932.
Northwestern University Medical School, May 24, 1932.

Iowa

Chairman: E. Witschi. Secretary: E. G. Gross. Members: 33.
Meetings: State University of Iowa, November 10, 1931.
State University of Iowa, January 26, 1932.
State University of Iowa, April 19, 1932.

Minnesota

Chairman: F. H. Scott. Secretary: F. H. Scott. Members: 49.
Meetings: University of Minnesota Medical School, October 21, 1931.
University of Minnesota Medical School, November 18, 1931.
University of Minnesota Medical School, December 16, 1931.
University of Minnesota Medical School, January 20, 1932.
University of Minnesota Medical School, April 20, 1932.
University of Minnesota Medical School, May 25, 1932.

Missouri

Chairman: M. S. Fleisher. Secretary: D. P. Barr. Members: 50.
Meetings: Washington University School of Medicine, November 11, 1931.
Washington University School of Medicine, February 10, 1932.
St. Louis University School of Medicine, March 9, 1932.
St. Louis University School of Medicine, May 11, 1932.

New York

Chairman: A. R. Dochez. Secretary: A. J. Goldforb. Members: 670.
Meetings: New York Academy of Medicine, October 21, 1931.
New York Academy of Medicine, November 18, 1931.
New York Academy of Medicine, December 16, 1931.
New York Academy of Medicine, January 20, 1932.
New York Academy of Medicine, February 17, 1932.
New York Academy of Medicine, March 16, 1932.
New York Academy of Medicine, April 20, 1932.
New York Academy of Medicine, May 18, 1932.

Pacific Coast

Chairman: W. Ophüls. Secretary: T. D. Beckwith. Members: 90.
Meetings: Stanford University, October 17, 1931.
Stanford University Medical School, December 17, 1931.
University of California Medical School, February 18, 1932.
University of California, April 23, 1932.

Peiping (China)

Chairman: C. M. Van Allen. Secretary: S. H. Liu. Members: 27.
Meetings: Peiping Union Medical College, October 22, 1931.
Peiping Union Medical College, January 29, 1932.
Peiping Union Medical College, April 28, 1932.

Southern

Chairman: W. H. Harris. Secretary: Harold Cummins. Members: 31.
Meetings: Tulane University, November 13, 1931.
Tulane University, January 29, 1932.
Tulane University, April 8, 1932.
Tulane University, May 20, 1932.

Western New York

Chairman: W. O. Fenn. Secretary: E. F. Adolph. Members: 47.

Meetings: Cornell University, October 17, 1931.
University of Buffalo, December 12, 1931.
University of Rochester Medical School, February 13, 1932.
Syracuse University Medical School, June 22, 1932.

Wisconsin

Chairman: L. J. Cole. Secretary: F. L. Hisaw. Members: 25.

DEATHS OF MEMBERS

The Council records with regret the deaths of the following members: F. Schwyzer and A. S. Warthin.

GIFTS

The Secretary wishes to acknowledge, with the thanks of the Council, the gift of back numbers from L. Baumann, H. F. Blum, M. M. Brooks, A. F. Coca, M. Cohen, W. Coleman, H. S. Forbes, A. B. D. Fortuyn, H. J. Fry, E. Holman, F. C. Koch, S. F. Light, E. S. London, J. F. Lyman, D. I. Macht, E. M. MacKay, C. S. Mudge, W. L. Palmer, W. W. Palmer, G. J. Pierce, L. F. Pollock, F. H. Pratt, N. K. Rakestraw, G. C. Robinson, M. S. Rose, G. G. Scott, E. S. Sevringhaus, K. Suguira, R. Tunnicliff, I. E. Wallin, C. K. Weichert, J. J. Willaman, R. R. Williams, R. M. Yerkes, and the Library, Harvard Medical School.

PROCEEDINGS

Average length of papers, 1.8 pp.
Preliminary manuscripts, 62%; complete manuscripts, 38%.

A. J. GOLDFORB,
Secretary.

TREASURER'S REPORT

April 1, 1931 to April 1, 1932

Receipts

Balance on Hand, April 1, 1931		$ 3,801.58
Income, 1931-32:		
Dues	$ 4,492.44	
Reprints	2,858.33	
Excess Space	1,676.09	
Cuts	401.78	
Subscriptions	2,561.39	
Back Numbers Sold	578.20	
Bank Interest and Miscellaneous	25.66	
Total Annual Income		$12,593.89
Total Cash Available April 1, 1931-April 1, 1932		$16,395.47

Disbursements

Publication Cost of Proceedings:		
Printing	$ 5,898.40	
Reprints	3,168.48	
Cuts	454.18	
		$ 9,521.06
Administrative Expenses:		
Office Supplies, Postage and Telegrams	$ 367.89	
Salaries	2,100.00	
Storage and Insurance	63.85	
Miscellaneous	41.92	
Overpaid	18.75	
		$ 2,592.41
Total Annual Disbursements		$12,113.47
Surplus Transferred to Special Surplus Fund		1,289.00
Cash Balance, April 1, 1932		2,993.00
Total		$16,395.47

Summary

Income (net)	$12,593.89
Disbursements (net)	12,113.47
Surplus for year	$ 480.42

Funds

Endowment Fund

April 1, 1931	$12,666.05	
Contributions to April 1, 1932	5.00	
Interest to April 1, 1932	668.66	
Total		$13,339.71

Invested in N. Y. Title and Mortgage Co.	$10,000.00	
Invested in R. R. Coop. Bldg. and Loan	3,339.71	
		$13,339.71

Special Surplus Fund

April 1, 1931	$ 5,661.53	
Annual Surplus Transferred to Fund	1,289.00	
Interest to April 1, 1932	287.41	
Total		$ 7,237.94

Invested in Title Guarantee and Trust Co.	$ 2,900.00	
Invested in R. R. Coop. Bldg. and Loan	4,337.94	
		$ 7,237.94

Life Membership Fund

Invested in R. R. Coop. Bldg. and Loan		$ 75.00

MEMO

Bills payable	None
Bills receivable	$ 1,369.90

A. J. GOLDFORB,
Treasurer.

Auditors' Report

We have examined the Treasurer's report and have verified the statements therein against the books of the Society. We congratulate Doctor Goldforb and his secretary, Miss McCabe, for the admirable way in which they are conducting the office of the Treasurer.

(Signed) ROBERT CHAMBERS,
IRVINE H. PAGE.

April 19, 1932.

MEMBERS' LIST (Alphabetical)

HONORARY MEMBERS

Councilman, William T.	Harvard University
Halliburton, W. D.	London, England
Howell, William H.	Johns Hopkins University
Lombard, Warren P.	University of Michigan
Porter, William	Harvard University
Reichert, E. T.	University of Pennsylvania
Richet, Charles	Paris, France
Schaffer, E. S.	Edinburgh, Scotland
Von Muller, Friedrich	Munich, Germany
Welch, W. H.	Johns Hopkins University

MEMBERS

Abbott, Alexander C.	University of Pennsylvania
Abel, John J.	Johns Hopkins University
Adams, A. Elizabeth	Mount Holyoke College
Addis, Thomas	Medical School, Stanford University
Adler, Herman M.	Berkeley, California
Adolph, E. F.	University of Rochester Medical School
Albus, William R.	American Type Culture Collection, Chicago
Alexander, Harry L.	Washington University
Allen, Bennet M.	University of California
Allen, Edgar	University of Missouri
Allen, Ezra	N. Y. Homeopathic Medical College
Allen, William F.	University of Oregon
Alsberg, Carl L.	Stanford University
Alt, Howard L.	Northwestern University Medical School
Alvarez, Walter C.	Mayo Clinic
Amberg, Samuel	Mayo Clinic
Amberson, W. R.	University of Tennessee Medical School
Amoss, Harold L.	Duke University Hospital
Anderson, H. H.	University of California Medical School
Anderson, John E.	University of Minnesota
Anderson, John F.	E. R. Squibb & Son
Anderson, Rudolph J.	Yale University
Andrews, Edmund	University of Chicago
Anson, Mortimer L.	Rockefeller Institute, Princeton, N. J.
Arnold, Lloyd	University of Illinois
Aronson, J. D.	Henry Phipps Institute
Asher, Leon	Berne, Switzerland
Ashman, Richard	Louisiana State Univ. Medical Center
Atchley, D. W.	Presbyterian Hospital, New York City

Atwell, Wayne J.	University of Buffalo, N. Y.
Aub, Joseph C.	Huntington Memorial Hospital, Boston
Auer, John	St. Louis University
Austin, J. Harold	University of Pennsylvania
Austin, W. C.	Loyola University
Avery, O. T.	Rockefeller Institute, N. Y. City

Babkin, Boris P.	McGill University
Bachem, Albert	University of Illinois Medical College
Baehr, George	Mt. Sinai Hospital, N. Y. City
Bagg, Halsey J.	Memorial Hospital, N. Y. City
Bailey, Cameron V.	N. Y. Post-Graduate Medical School
Baitsell, George A.	Yale University
Bakwin, Harry	N. Y. University and Bellevue Medical College
Baldridge, Clarence W.	State University of Iowa
Baldwin, W. Manning	Albany Medical College
Ball, G. H.	University of California, Los Angeles
Balls, A. K.	Bremen, Germany
Banta, A. M.	Brown University
Banting, Frederick G.	University of Toronto
Barach, Alvin L.	College of Physicians and Surgeons
Barber, W. Howard	New York University Med. School
Barbour, Henry G.	Yale University
Bardeen, Charles R.	University of Wisconsin
Barlow, Orphus W.	Western Reserve University
Barnett, George D.	Stanford University
Barr, David P.	Washington University
Barron, E. S. G.	University of Chicago
Bass, Charles	Tulane University
Bast, T. H.	University of Wisconsin
Bauer, J. H.	Rockefeller Institute
Bauman, Louis	Presbyterian Hospital, N. Y. City
Baumann, E. J.	Montefiore Hospital, N. Y. City
Baumberger, J. Percy	Stanford University
Bayne-Jones, S.	University of Rochester
Bazett, H. C.	University of Pennsylvania
Beard, H. H.	Louisiana State Univ. Medical Center
Becker, E. R.	Iowa State College
Beckwith, T. D.	University of California
Behre, Jeannette A.	Cornell University Medical College
Belding, David L.	Boston University
Bellamy, Arthur W.	University of California, Los Angeles
Benedict, S. R.	Cornell University Medical College, N. Y. City
Benton, Anne G.	Vassar College
Berg, B. N.	Columbia University
Berg, William N.	Berg Biological Laboratory, N. Y. City
Bergeim, Olaf	University of Illinois
Bergey, David H.	National Drug Co., Philadelphia
Berglund, Hilding	University of Minnesota
Bernhard, Adolph	Lenox Hill Hospital, N. Y. City

Beutner, R.	University of Louisville
Bieter, Raymond N.	University of Minnesota
Bills, C. E.	Mead, Johnson and Co., Evansville, Ind.
Bing, Franklin C.	Western Reserve University
Binger, Carl A. L.	Rockefeller Institute, N. Y. City
Birkhaug, Konrad E.	University of Rochester
Bishop, George H.	Webster Groves, Mo.
Bishop, Katharine S.	University of California
Blackfan, K. D.	Harvard Medical School
Blair, John E.	Hospital for Joint Diseases, N. Y. City
Blake, F. G.	Yale University
Blakesley, Albert F.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Blatherwick, Norman R.	Metropolitan Life Insurance Co., New York City
Blau, Nathan F.	Cornell University Medical College
Blinks, L. R.	Rockefeller Institute
Bliss, Sidney	Tulane University
Bloom, William	University of Chicago
Bloomfield, A. L.	Stanford University Medical School
Bloor, W. R.	University of Rochester
Blum, Harold F.	University of California
Blumgart, H. L.	Beth Israel Hospital, Boston
Bodansky, A.	New York City
Bodansky, Meyer	John Sealy Hospital, Galveston, Texas
Bodine, J. H.	State University of Iowa
Bodo, Richard	N. Y. University and Bellevue Med. College
Bollman, Jesse L.	Mayo Clinic
Boor, Alden K.	University of Chicago
Boothby, Walter M.	Kahler Hospital, Rochester, Minn.
Boots, Ralph H.	Presbyterian Hospital, New York City
Bowen, B. D.	Buffalo General Hospital
Boyd, Julian D.	State University of Iowa
Boyd, Theo. E.	Loyola University
Boyden, E. A.	University of Minnesota Medical School
Bradley, H. C.	University of Wisconsin
Brand, Erwin	New York State Psychiatric Institute
Branham, Sara E.	Hygienic Laboratory, Washington, D. C.
Brewer, Robert K.	Syracuse University
Briggs, A. P.	St. Louis University
Bronfenbrenner, J.	Washington University
Bronk, D. W.	University of Pennsylvania
Brooks, Clyde	Louisiana State University Medical Center
Brooks, Harlow	New York University
Brooks, Matilda M.	University of California
Brooks, S. C.	University of California
Broun, G. O.	St. Mary's Infirmary, St. Louis
Brown, E. D.	University of Minnesota
Brown, J. Howard	Johns Hopkins University
Brown, John B.	Ohio State University
Brown, L. A.	George Washington University
Brown, Wade H.	Rockefeller Institute, N. Y. City

Buchanan, Robert E.	Iowa State College
Buchbinder, W. C.	Michael Reese Hospital, Chicago
Bulger, H. A.	Washington University
Bull, C. G.	Johns Hopkins University
Bullowa, J. G. M.	Harlem Hospital, N. Y. City
Bunting, C. H.	University of Wisconsin
Bunting, R. W.	University of Michigan
Burch, John C.	Vanderbilt University Med. School
Burget, G. E.	University of Oregon Medical School
Burky, Earl L.	Johns Hopkins Hospital
Burnett, Theodore C.	University of California
Burns, Robert K., Jr.	University of Rochester
Burr, Harold S.	Yale University
Burrows, M. T.	Pasadena, Calif.
Byrne, Joseph	Fordham University
Calkins, Gary N.	Columbia University
Cannan, Robert K.	N. Y. University and Bellevue Med. College
Cannon, Paul R.	University of Chicago
Cannon, Walter B.	Harvard Medical School
Carlson, A. J.	University of Chicago
Carmichael, E. B.	University of Alabama School of Medicine
Carruthers, Albert	Peiping Union Medical College
Carter, Edward P.	Johns Hopkins Hospital
Casey, Albert E.	Rockefeller Institute
Cash, James R.	University of Virginia
Cattell, McKeen	Cornell University Medical College
Caulfield, A. H.	University of Toronto
Cecil, R. L.	Cornell University Medical College
Chace, Arthur F.	N. Y. Post-Graduate Medical College
Chaikoff, I. L.	University of California
Chambers, Robert	New York University
Chambers, William H.	Cornell University Medical College
Chang, Hsi Chun	Peiping Union Medical College
Chang, Hsiao-Chien	Peiping Union Medical College
Cheer, S. N.	Peiping Union Medical College
Chen, K. K.	Eli Lilly and Co., Indianapolis
Chidester, F. E.	University of West Virginia
Child, C. M.	University of Chicago
Chittenden, R. H.	Yale University
Chou, T.	Peiping Union Medical College, China
Christian, Henry A.	Peter Bent Brigham Hospital
Christman, Adam A.	University of Michigan
Churchman, John W.	Cornell University Medical School
Clark, Guy W.	Lederle Lab., Pearl River, N. Y.
Clark, P. F.	University of Wisconsin
Clarke, Hans T.	College of Physicians and Surgeons
Claussen, S. W.	Strong Memorial Hospital, Rochester, N. Y.
Clowes, G. H. A.	Eli Lilly and Co., Indianapolis, Indiana
Coca, A. F.	Cornell University Medical College

Coghill, G. E.	Wistar Institute
Cohen, Barnett	Johns Hopkins Medical School
Cohen, Martin	N. Y. Post-Graduate Medical School
Cohn, A. E.	Rockefeller Institute, N. Y. City
Cohn, Isidore	New Orleans, La.
Cole, L. J.	University of Wisconsin
Cole, Rufus I.	Rockefeller Institute, N. Y. City
Cole, William H.	Rutgers University
Coleman, Warren	New York University Medical College
Collens, William S.	Brooklyn Jewish Hospital
Collett, Mary E.	Western Reserve University
Collier, William D.	St. Louis University
Collins, H. H.	University of Pittsburgh
Collip, J. B.	McGill University
Conklin, E. G.	Princeton University
Connor, Charles L.	University of California Medical School
Cook, Donald H.	School of Tropical Medicine, San Juan, Porto Rico
Cook, S. F.	University of California
Cooke, J. V.	Washington University
Coombs, Helen C.	New York Homeopathic Medical College
Copenhaver, W. M.	Columbia University
Cori, Carl F.	Washington University
Corley, Ralph C.	Purdue University
Corner, George W.	University of Rochester
Corper, H. J.	National Jewish Hospital, Denver, Colo.
Coulter, Calvin B.	Columbia University
Cowdry, E. V.	Washington University
Cowgill, George R.	Yale University
Cowie, D. M.	University of Michigan
Craig, C. F.	Tulane University
Crampton, C. Ward	N. Y. Post-Graduate Medical School
Crandall, L. A., Jr.	Northwestern University
Crile, George W.	Western Reserve University
Crohn, Burrill B.	Mt. Sinai Hospital, N. Y. City
Crozier, W. J.	Harvard University
Cruickshank, E. W. H.	Dalhousie University, Halifax
Csonka, F. A.	U. S. Bureau Chemistry, Washington, D. C.
Cullen, Glenn E.	Children's Hospital, Cincinnati, O.
Cummins, Harold	Tulane University
Cunningham, R. S.	Vanderbilt University
Curtis, G. M.	University of Chicago
Curtis, Maynie R.	Columbia University
Cushing, Harvey W.	Harvard Medical School
Cutler, Elliott C.	Western Reserve University

Dack, Gail M.	University of Chicago
Dakin, H. D.	Ossining, N. Y.
Danforth, Charles H.	Stanford University
Daniel, J. Frank	University of California
Daniels, Amy L.	University of Iowa

Daniels, Farrington	University of Wisconsin
Danzer, Charles S.	N. Y. Homeopathic Medical College
Davenport, C. B.	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Davenport, H. A.	Northwestern University Medical School
Davies, H. Whitridge	The University, Sydney, Australia
Davis, D. J.	Wilmette, Illinois
Davis, Nelson C.	Rockefeller Foundation
Dawson, James A.	College of the City of New York
Dawson, W. T.	University of Texas Med. School
Day, A. A.	Northwestern University Medical School
De Eds, Floyd	Stanford University Medical School
DeGraff, A. C.	N. Y. University Medical School
Derick, C. L.	Peter Bent Brigham Hospital, Boston
Detwiler, S. R.	Columbia University
Deuel, Harry J., Jr.	University of Southern California Medical School
Dickson, E. C.	Stanford University Medical School
Dienes, Louis	Massachusetts General Hospital, Boston
Dieuaide, Francis R.	Peiping Union Medical College, China
Doan, Charles A.	Ohio State University
Dochez, A. R.	Presbyterian Hospital, N. Y. City
Dock, William	Stanford University
Doisy, Edward A.	St. Louis University
Dolley, W. L., Jr.	University of Buffalo
Donaldson, H. H.	Wistar Institute, Philadelphia
Donaldson, J. C.	University of Pittsburgh
Dooley, M. S.	Syracuse University
Downs, W. G., Jr.	Chicago, Ill.
Drabkin, D. L.	University of Pennsylvania
Dragstedt, Carl A.	Northwestern University
Dragstedt, Lester R.	University of Chicago
Draper, George W.	Columbia University
Draper, John W.	New York City
Dresbach, M.	Albany Medical College
Drury, D. R.	Harvard Medical School
Dubin, Harry E.	Metz Laboratories, N. Y. City
DuBois, E. F.	Cornell University Medical College
Duggar, B. M.	University of Wisconsin
Dunn, L. C.	Columbia University
Dunn, Max	University of California, Los Angeles
Durrant, Edwin P.	Ohio State University
Dusser de Barenne, J. G.	Yale University
Dutcher, R. Adams	Pennsylvania State College
Duval, C. W.	Tulane University
Du Vigneaud, Vincent	University of Illinois
Dye, Joseph A.	Cornell University Medical College
Eberson, Frederick	University of California Medical School
Ecker, E. E.	Western Reserve University
Eckles, C. H.	University of Minnesota
Eckstein, Henry C.	University of Michigan

Eddy, Walter H.	Columbia University
Edmunds, C. W.	University of Michigan
Edwards, D. J.	Cornell University Medical College
Edwards, John G.	University of Buffalo
Eggston, Andrew A.	Manhattan Eye, Ear, Nose and Throat Hospital, N. Y. City
Eisberg, Harry B.	N. Y. University and Bellevue Medical College
Ellis, Max M.	University of Missouri
Elsberg, Charles A.	Neurological Institute, N. Y. City
Elser, W. J.	Cornell University Medical College
Emery, F. E.	University of Buffalo
Engle, E. T.	Columbia University
Epstein, A. A.	Mt. Sinai Hospital, N. Y. City
Erdmann, Rhoda	University of Berlin, Germany
Erlanger, Joseph	Washington University
Ernstene, Arthur C.	Harvard Medical School
Essex, Hiram E.	Mayo Clinic
Evans, Herbert M.	University of California
Ewing, James	Cornell University Medical College
Eyster, J. A. E.	University of Wisconsin
Faber, Harold K.	Stanford University Medical School
Fahr, George	University of Minnesota
Falk, I. S.	Washington, D. C.
Falk, K. George	Harriman Laboratories, N. Y. City
Falls, Frederick H.	University of Illinois
Famulener, L. W.	St. Luke's Hospital, N. Y. City
Farmer, Chester	Northwestern University
Faust, Ernest C.	Tulane University
Fenn, Wallace O.	University of Rochester Medical School
Ferry, R. M.	Harvard University
Field, Cyrus W.	New York City
Field, John, II	Stanford University
Fine, M. S.	Battle Creek, Mich.
Fischer, Albert	Kopenhagen, Denmark
Fischer, Martin H.	University of Cincinnati
Fishberg, Ella H.	Beth Israel Hospital, New York City
Fisher, Nelson F.	St. Luke's Hospital, Chicago
Fitch, C. P.	University of Minnesota
Fitz, Reginald	Peter Bent Brigham Hospital
Fitzgerald, J. G.	University of Toronto
Fleisher, Moyer S.	St. Louis University
Flexner, Simon	Rockefeller Institute, N. Y. City
Flinn, Frederick B.	Columbia University
Florence, Laura	New York Homeopathic Medical School
Fluhmann, Charles F.	Stanford University Medical School
Forbes, Henry	Milton, Mass.
Forkner, Claude E.	Boston City Hospital
Fortuyn, A. B. D.	Peiping Union Medical College
Foster, Goodwin L.	Columbia University

Foster, Nellis B.	New York Hospital, N. Y. City
Frank, Robert T.	Mt. Sinai Hospital
Frazier, Chester N.	Bluffton, Indiana
Fred, E. B.	University of Wisconsin
Freedman, Louis	Metz Laboratory
Freeman, R. G., Jr.	Columbia University
Freund, Jules	Henry Phipps Institute, Philadelphia
Fridericia, L. S.	University of Copenhagen, Denmark
Frobisher, Martin, Jr.	Rio de Janiero, Brazil
Fry, Henry J.	New York University
Fulmer, Ellis I.	Iowa State College
Fulton, John F.	Yale Medical School
Funk, Casimir	Paris, France
Furth, Jacob	Henry Phipps Institute, Philadelphia
Gaebler, O. H.	Henry Ford Hospital, Detroit
Gage, I. M.	Tulane University
Gager, C. Stuart	Brooklyn Botanic Garden, Brooklyn, N. Y.
Gahl, Rudolph	University of California
Gamble, James L.	Harvard University
Garbat, Abraham L.	Lenox Hill Hospital, N. Y. City
Gardner, Leroy U.	Saranac Lab. for Study of Tuberculosis
Garrey, Walter E.	Vanderbilt University
Gaskell, John F.	Cambridge, England
Gasser, Herbert S.	Washington University
Gates, Frederick L.	Cambridge, Mass.
Gay, F. P.	Columbia University
Geiling, E. M. K.	Johns Hopkins Medical School
Gelfan, Samuel	University of Alberta, Canada
Gellhorn, Ernst	University of Oregon
Gerard, R. W.	University of Chicago
Gesell, Robert A.	University of Michigan
Gettler, A. O.	University and Bellevue Medical College
Geyelin, H. R.	Columbia University
Gibson, R. B.	University of Iowa
Gies, William J.	Columbia University
Gilbert, E. M.	University of Wisconsin
Gilchrist, Francis G.	Pomona College
Gilligan, Dorothy R.	Beth Israel Hospital, Boston
Githens, T. S.	Mulford Company, Philadelphia, Pa.
Givens, Maurice H.	Northwestern Yeast Co., Chicago
Glaser, Otto	Amherst College
Gold, Harry	Cornell University Medical College
Goldberg, S. A.	Presbyterian Hospital, Newark, N. J.
Goldblatt, Harry	Western Reserve University
Goldbloom, A. Allen	Beth Israel Hospital, N. Y. City
Goldforb, A. J.	College of the City of New York
Goldschmidt, Samuel	University of Pennsylvania
Gortner, R. A.	University of Minnesota
Gottesman, J. Marmorston	Montefiore Hospital, N. Y. City

Gould, Harley N.	Tulane University
Graham, Evarts A.	Washington University
Graham, Helen T.	Washington University
Graves, William W.	St. Louis University
Gray, Samuel H.	Jewish Hospital, St. Louis, Mo.
Green, Robert G.	University of Minnesota
Greenberg, David N.	University of California
Greene, Carl H.	Mayo Clinic
Greenwald, Isidor	N. Y. University and Bellevue Med. College
Greer, J. R.	University of Chicago
Gregory, Louise H.	Barnard College, Columbia University
Greisheimer, Esther M.	University of Minnesota
Griffith, Fred R., Jr.	University of Buffalo
Griffith, Wendell H.	St. Louis University
Gross, Erwin G.	State University of Iowa
Gross, Louis	Mt. Sinai Hospital, N. Y. City
Gruber, Charles M.	Washington University
Guberlet, J. E.	University of Washington
Gunn, Francis D.	Northwestern University Med. School
Guenther, A. E.	University of Nebraska
Gurchot, Charles	Eldridge, California
Gustavson, R. G.	University of Denver
Guthrie, C. C.	University of Pittsburgh
Guy, Ruth A.	Peiping Union Medical College
Guyer, Michael F.	University of Wisconsin
Hadley, Philip	University of Michigan
Hafkesbring, H. Roberta	Woman's Medical College, Philadelphia
Hagan, William Arthur	Cornell University
Hall, Ernest M.	University of Southern California Med. School
Halsey, John	Tulane University
Halsey, Robert H.	N. Y. Post-Graduate Medical School
Hamburger, W. W.	Rush Medical College
Hamilton, B. K.	Chicago, Illinois
Hammer, B. W.	Iowa State College
Hanger, Franklin M.	Presbyterian Hospital, N. Y. City
Hanke, Martin E.	University of Chicago
Hannon, R. R.	Peiping Union Medical College
Hansman, G. H.	State University of Iowa
Hanson, Frank B.	Washington University
Hanzlik, P. J.	Stanford University Medical School
Hardesty, Irving	Tulane University
Harris, Isaac F.	Tuckahoe, N. Y.
Harris, Reginald G.	Cold Spring Harbor, N. Y.
Harris, William H.	Tulane University
Harrison, R. G.	Yale University
Harrop, George A., Jr.	Johns Hopkins University
Harrow, Benjamin	College of the City of New York
Hart, Edwin B.	University of Wisconsin
Hart, George H.	University Farm, Davis, California

Hartman, F. A.	University of Buffalo
Hartwell, John A.	Cornell University Medical College
Harvey, E. Newton	Princeton University
Harvey, Samuel C.	Yale University
Hastings, A. Baird	University of Chicago
Hastings, E. G.	University of Wisconsin
Hatcher, R. A.	Cornell University Medical College
Hathaway, Edward S.	Tulane University
Hawk, P. B.	New York City
Hawkins, James A.	U. S. Navy Yards, Washington, D. C.
Hayden, Charles E.	Cornell University
Haythorn, Samuel R.	Singer Research Lab., Pittsburgh, Pa.
Heft, Hattie L.	Columbia University
Heidelberger, Michael	Presbyterian Hospital, New York City
Heilbrunn, L. V.	University of Pennsylvania
Heinbecker, Peter	Washington University
Helff, O. M.	New York University
Helmholz, Henry R.	Mayo Clinic
Hench, Philip S.	Mayo Clinic
Hendrix, B. M.	University of Texas
Henrici, Arthur T.	University of Minnesota
Herrmann, G. R.	John Sealy Hospital, Galveston, Texas
Hertzman, A. B.	St. Louis University
Hess, Alfred F.	College of Physicians and Surgeons
Heymans, J. F.	U. de Gand, Belgium
Higgins, George M.	Mayo Clinic
Hill, Eben C.	Johns Hopkins University
Hill, Samuel E.	Rockefeller Institute
Himwich, H. E.	Yale University
Hines, H. M.	State University of Iowa
Hinman, Frank	University of California Med. School
Hinrichs, Marie Agnes	University of Chicago
Hinsey, J. C.	Stanford University
Hinshaw, H. C.	Upper Darby, Pa.
Hirschfelder, Arthur	University of Minnesota
Hisaw, F. L.	University of Wisconsin
Hitchcock, Fred A.	Ohio State University
Hobmaier, Michael	University of California
Hoeppli, R. J. C.	Peiping Union Medical College
Hoffman, George L.	Allegheny County Hospital, Pittsburgh, Pa.
Hollander, Franklin	N. Y. Homeopathic Medical College
Holm, George E.	Department of Agriculture, Washington, D. C.
Holman, Emil	Stanford University
Holman, W. L.	University of Toronto
Holmes, S. J.	University of California
Holt, L. Emmett, Jr.	Johns Hopkins University
Hooker, Davenport	University of Pittsburgh
Hooker, Sanford B.	Boston University
Hooper, Charles W.	Rensselaer, N. Y.
Hopkins, J. Gardner	Columbia University

Horsley, J. Shelton	St. Elizabeth's Hospital, Richmond, Va.
Hou, Hsiang-Chuan	Peiping Union Medical College
Howard, Harvey J.	Washington University
Howe, Paul E.	Department of Agriculture, Washington, D. C.
Howell, K. M.	Michael Reese Hospital
Howland, Ruth B.	New York University
Hubbard, Roger S.	Buffalo General Hospital
Huber, G. Carl	University of Michigan
Hudson, N. Paul	University of Chicago
Hughes, Thomas P.	Rockefeller Institute
Humphrey, R. R.	University of Buffalo
Hunt, Reid	Harvard University
Huntoon, F. M.	Mulford Co., Glenolden, Pa.
Hurwitz, Samuel	Stanford Medical School
Hussey, Raymond	Yale University

Irwin, Marion	Rockefeller Institute
Isaacs, Raphael	Simpson Memorial Institute, Ann Arbor
Ivy, Andrew C.	Northwestern University

Jackson, C. M.	University of Minnesota
Jackson, D. E.	University of Cincinnati
Jackson, Henry, Jr.	Boston City Hospital
Jacobs, M. H.	University of Pennsylvania
Jacobs, Walter A.	Rockefeller Institute, N. Y. City
Jacobsen, Victor C.	Albany Medical College
Jaffe, Henry L.	Hospital for Joint Diseases, N. Y. City
Jaffe, Richard H.	University of Illinois
Jeans, Philip C.	University of Iowa
Jennings, H. S.	Johns Hopkins University
Jobling, J. W.	Columbia University
Johlin, J. M.	Vanderbilt University School of Medicine
Johns, Foster M.	Tulane University
Johnson, C. A.	University of Chicago
Johnson, C. C.	University of Utah
Johnson, T. B.	Yale University
Jolliffe, Norman H.	N. Y. University and Bellevue Med. College
Jonas, Leon	University of Pennsylvania
Jones, Frederick S.	Rockefeller Institute, Princeton, N. J.
Jordan, Edwin	University of Chicago
Jordan, H. E.	University of Virginia
Jorstad, L. H.	Barnard Free Skin and Cancer Hospital, St. Louis, Mo.
Joslin, E. P.	Boston, Mass.
Julianelle, Louis A.	Washington University
Jung, F. T.	Northwestern University Medical School
Jungeblut, Claus	College of Physicians and Surgeons

Kahn, Morris H.	Beth Israel Hospital, N. Y. City
Kahn, Morton C.	Cornell Medical College, N. Y. City
Kahn, R. L.	University Hospital, Ann Arbor, Mich.

Kamm, Oliver	Parke, Davis & Co., Detroit
Karsner, H. T.	Western Reserve University
Kast, Ludwig	N. Y. Post-Graduate Medical School
Katayama, I.	St. Luke's International Hospital, Tokyo
Katz, L. N.	Michael Reese Hospital, Chicago
Keefer, Chester S.	Boston City Hospital
Keeton, Robert W.	University of Illinois
Keitt, G. W.	University of Wisconsin
Keller, Allen D.	University of Alabama Medical School
Kellogg, W. H.	University of California
Kendall, Arthur I.	Northwestern University Medical School
Kendall, E. C.	Mayo Clinic, Minn.
Kerr, William J.	University of California Medical School
Kessel, John F.	University of Southern California
Key, John A.	Washington University
Killian, J. A.	N. Y. Post-Graduate Medical School
King, Helen D.	Wistar Institute, Philadelphia
Kinsella, Ralph A.	St. Louis University
Kirkbride, Mary B.	N. Y. State Dept. of Health, Albany, N. Y.
Kleiner, I. S.	N. Y. Homeopathic Medical School
Kleitman, Nathaniel	University of Chicago
Kligler, I. J.	Hebrew University, Palestine
Kline, B. S.	Mt. Sinai Hospital, Cleveland, Ohio
Klotz, Oskar	University of Toronto
Knowlton, Frank P.	Syracuse University
Knudson, Arthur	Albany Medical College
Kober, Philip A.	G. D. Searle and Co., Chicago
Koch, Elizabeth	University of Illinois
Koch, Fred C.	University of Chicago
Koch, Mathilda L.	Greenwich, Conn.
Koehler, Alfred E.	Santa Barbara Cottage Hospital, Calif.
Kofoid, Charles A.	University of California
Kohn, Jerome L.	Mt. Sinai Hospital, New York City
Kolmer, John A.	University of Pennsylvania
Kopeloff, Nicholas	Psychiatric Institute, N. Y. City
Koppányi, Theodore	Georgetown University Medical School
Korns, Horace M.	State University of Iowa
Korns, John H.	Olean, N. Y.
Koser, Stewart	University of Chicago
Kramer, Benjamin	Brooklyn Jewish Hospital
Krasnow, Frances	Columbia University
Krizenecky, J.	Brno, Czechoslovakia
Krueger, A. P.	University of California
Krumbhaar, E. B.	University of Pennsylvania
Kruse, Theophile K.	University of Pittsburgh
Kugelmass, I. Newton	New York City
Kunde, Margaret M.	University of Chicago
Kunitz, Moses	Rockefeller Institute, Princeton, N. J.
Kuntz, Albert	St. Louis University
Kurotchkin, T. J.	Peiping Union Medical College

Ladd, William S.	Presbyterian Hospital
Lamar, R. V.	Milledgeville State Hospital, Georgia
Lambert, R. A.	Rockefeller Foundation
Lamson, Paul D.	Vanderbilt University
Lamson, Robert W.	Los Angeles, Calif.
Lancefield, D. E.	Columbia University
Landers, Carney	Psychiatric Institute, N. Y. City
Landsteiner, Karl	Rockefeller Institute, N. Y. City
Langstroth, Lovell	San Francisco, California
Larsell, Olaf	University of Oregon
Larson, Edward	Temple University
Larson, J. A.	Institute for Juvenile Research, Chicago
Larson, W. P.	University of Minnesota
Lashley, K. S.	University of Chicago
Lathrop, Carl O.	University of Buffalo
Laughlin, H. H.	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Laurens, Henry	Tulane University
Leake, C. D.	University of California Medical School
Leake, J. P.	Hygienic Laboratory, Washington, D. C.
Lee, Ferdinand C.	Johns Hopkins University
Lee, Frederic S.	Columbia University
Lee, Milton O.	Harvard University
Leiter, Louis	University of Chicago
Leonard, C. S.	Burroughs Wellcome and Co.
Leonard, Veader	Johns Hopkins University
Levene, P. A.	Rockefeller Institute, N. Y. City
Levin, Isaac	New York City
Levine, Max	Iowa State College
Levine, Michael	Montefiore Hospital, N. Y. City
Levine, Samuel Z.	Cornell University Medical College
Levine, Victor E.	Creighton University
Levinson, Samuel A.	University of Illinois
Levy, Robert L.	Presbyterian Hospital, New York City
Lewis, Howard B.	University of Michigan
Lewis, Robert C.	University of Colorado
Liddell, H. S.	Cornell University
Lieb, C. C.	Columbia University
Light, S. F.	University of California
Lillie, Frank R.	University of Chicago
Lillie, Ralph S.	University of Chicago
Lim, C. E.	Peiping Union Medical College
Lim, Robert K.	Peiping Union Medical College China
Lindstrom, E. W.	Iowa State College
Ling, Schmorl M.	Peiping Union Medical College China
Linton, Richard W.	College of Physicians and Surgeons
Lipman, Charles B.	University of California
Little, C. C.	Bar Harbor, Maine
Liu, Shih-hao	Peiping Union Medical College
Locke, A. P.	St. Luke's Hospital, Chicago
Loeb, Leo	Washington University

Loeb, Robert F.	Presbyterian Hospital, N. Y. City
Loebel, Robert O.	Cornell University Med. College
Loehwing, W. F.	University of Iowa
London, E. S.	Leningrad, U. S. S. R.
Long, Perrin H.	Johns Hopkins Hospital
Longcope, W. T.	Johns Hopkins University
Looney, J. M.	Worcester State Hospital, Mass.
Lorenz, William F.	University of Wisconsin
Lucas, William P.	University of California Med. School
Luck, J. Murray	Stanford University
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McQuarrie, Irvine	University of Minnesota
MacDougal, D. T.	Desert Laboratory, Tucson, Arizona
MacDowell, E. Carlton	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
MacKay, E. M.	Scripps Metabolic Clinic, San Diego, Calif.
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Meigs, E. B.	Dairy Division Exp. Station, Beltsville, Md.
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Miller, Joseph L.	St. Luke's Hospital, Chicago
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Ray, G. B.	Western Reserve University
Ray, Henry M.	South Side Hospital, Pittsburgh

Read, Bernard E.	Peiping Union Medical College, China
Read, J. Marion	Stanford University Medical School
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Richey, DeWayne G.	University of Pittsburgh
Richter, Maurice	Columbia University
Riddle, Oscar	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Ringer, A. I.	Montefiore Hospital, N. Y. City
Ringer, Michael	Montefiore Hospital, N. Y. City
Rivers, Thomas M.	Rockefeller Institute
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Robinson, Elliott	Antitoxin Laboratory, Boston
Robinson, G. Canby	Cornell University Medical College
Rogers, Fred T.	Baylor University
Rogoff, J. M.	Western Reserve University
Rohdenburg, George L.	Lenox Hill Hospital, N. Y. City
Romanoff, Alexis L.	Cornell University
Rose, Anton R.	Prudential Life Insurance Co., Newark, N. J.
Rose, Mary Swartz	Columbia University
Rose, William C.	University of Illinois
Rosenow, E. C.	Mayo Foundation
Rosenthal, Lazar	United Israel-Zion Hospital, Brooklyn
Ross, Victor	Department of Health, N. Y. City
Roth, George B.	George Washington University
Rothschild, M. A.	Mt. Sinai Hospital, N. Y. City
Rous, Peyton	Rockefeller Institute
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Russell, W. C.	N. J. Agricultural Experiment Station

Sabin, Florence R.	Rockefeller Institute
Sahyun, Melville	Santa Barbara, Calif.
Salant, William	Biological Laboratory, Cold Spring Harbor, N. Y.
Salle, A. J.	U. S. Dept. of Public Health, Honolulu
Salvesen, Harold A.	Rikshospitalet, Oslo, Norway
Sandburg, M.	Montefiore Hospital
Sanford, A. H.	Mayo Clinic
Sansum, W. D.	Santa Barbara Cottage Hospital
Sawyer, W. A.	Rockefeller Foundation
Scammon, R. E.	University of Minnesota

Schick, Béla	Mount Sinai Hospital, N. Y. City
Schlesinger, M. J.	Beth Israel Hospital, Boston
Schloss, Oscar M.	Cornell University Medical College
Schlutz, F. W.	University of Chicago
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Schmidt, Carl L. A.	University of California
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Schneider, Edward C.	Wesleyan University
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Schultz, O. T.	St. Francis Hospital, Evanston, Illinois
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Scott, R. W.	Western Reserve University
Scott, W. J. M.	University of Rochester
Sears, H. J.	University of Oregon
Seegal, Beatrice C.	College of Physicians and Surgeons
Senior, Harold D.	N. Y. University and Bellevue Medical College
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Shaffer, Philip A.	Washington University
Shaklee, A. O.	St. Louis University
Shannon, W. R.	University of Minnesota
Sharlit, Herman	Roosevelt Hospital, N. Y. City
Sharp, P. E.	Cornell University
Shear, M. J.	Harvard Medical School
Sheard, Charles	Mayo Clinic
Shelling, D. H.	Johns Hopkins Hospital
Sherman, H. C.	Columbia University
Sherman, Hartley E.	Bureau of Science, Manila, P. I.
Sherman, James M.	Cornell University
Sherwin, Carl P.	Fordham University
Shibley, Gerald S.	Lakeside Hospital, Cleveland
Shiple, George J.	University of Detroit
Shohl, Alfred T.	Western Reserve University
Shope, R. E.	Rockefeller Institute, Princeton, N. J.
Shwartzman, Gregory	Mt. Sinai Hospital
Sia, Richard H. P.	Peiping Union Medical College
Sickles, Grace M.	N. Y. State Department of Health
Simonds, J. P.	Northwestern University
Sinclair, R. G.	University of Rochester
Sittenfield, M. J.	Columbia University
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Smetana, Hans	Columbia University
Smith, Arthur H.	Yale University
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Smith, Clarence	Bergenfield, New Jersey
Smith, Erma A.	Iowa State College

Smith, Fred M.	University of Iowa
Smith, George H.	Yale University
Smith, Harry P.	State University of Iowa
Smith, Homer W.	N. Y. University and Bellevue Med. College
Smith, Lawrence W.	Cornell Medical College
Smith, Millard	Boston, Mass.
Smith, Philip E.	Columbia University
Smith, Theobald	Rockefeller Institute, Princeton, N. J.
Smyly, H. Joselyn	Shantung Christian University, China
Snell, A. M.	Mayo Clinic
Snyder, Franklin F.	Johns Hopkins University
Sollmann, Torald	Western Reserve University
Somogyi, Michael	Jewish Hospital, St. Louis, Mo.
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Soule, M. H.	University of Michigan
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Sponsler, O. L.	University of California, Los Angeles
Stakman, E. C.	University of Minnesota
Stark, Clifford N.	Cornell University
Stark, Mary B.	N. Y. Homeopathic Medical College, N. Y. City
Starkey, R. L.	Agricultural Exp. Station, New Brunswick, N. J.
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Tso, Ernest	Peiping Union Medical College
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Wadsworth, Augustus B.	N. Y. State Department of Health
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Wilhelmj, C. M.	Creighton University Medical School
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Youland, William E., Jr.	N. Y. Homeopathic Medical College
Young, C. C.	Department of Health, Mich.
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